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United States Patent [19]**Chandrashekar et al.**[11] **Patent Number:** **5,854,051**[45] **Date of Patent:** **Dec. 29, 1998**[54] **PARASITIC HELMINTH ASPARAGINASE PROTEINS, NUCLEIC ACID MOLECULES, AND USES THEREOF**[75] Inventors: **Ramaswamy Chandrashekar; Naotoshi Tsuji**, both of Fort Collins, Colo.[73] Assignees: **Heska Corporation; Colorado State University Research Foundation**, both of Fort Collins, Colo.[21] Appl. No.: **929,501**[22] Filed: **Sep. 15, 1997**[51] **Int. Cl.**⁶ **C12N 9/82; C12N 1/20; C07H 21/04**[52] **U.S. Cl.** **435/227; 435/228; 435/229; 435/252.3; 435/252.33; 435/320.1; 536/23.2; 536/23.4; 536/23.1**[58] **Field of Search** **435/227, 228, 435/229, 252.3, 252.33, 325, 320.1; 536/23.1, 23.2, 23.4, 24.3, 24.33**[56] **References Cited**
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[57] **ABSTRACT**

The present invention relates to: parasitic helminth asparaginase proteins; parasitic helminth asparaginase nucleic acid molecules, including those that encode such asparaginase proteins; antibodies raised against such asparaginase proteins; and compounds that inhibit parasitic helminth asparaginase activity. The present invention also includes methods to obtain such proteins, nucleic acid molecules, antibodies, and inhibitory compounds. Also included in the present invention are therapeutic compositions comprising such proteins, nucleic acid molecules, antibodies and/or inhibitory compounds as well as the use of such therapeutic compositions to protect animals from diseases caused by parasitic helminths.

9 Claims, No Drawings

**PARASITIC HELMINTH ASPARAGINASE
PROTEINS, NUCLEIC ACID MOLECULES,
AND USES THEREOF**

FIELD OF THE INVENTION

The present invention relates to parasitic helminth asparaginase nucleic acid molecules, proteins encoded by such nucleic acid molecules, antibodies raised against such proteins, and inhibitors of such proteins. The present invention also includes therapeutic compositions comprising such nucleic acid molecules, proteins, antibodies, inhibitors, and combinations thereof, as well as the use of these compositions to protect animals from diseases caused by parasitic helminths, such as heartworm disease.

BACKGROUND OF THE INVENTION

Parasitic helminth infections in animals, including humans, are typically treated by chemical drugs. One disadvantage with chemical drugs is that they must be administered often. For example, dogs susceptible to heartworm are typically treated monthly. Repeated administration of drugs, however, often leads to the development of resistant helminth strains that no longer respond to treatment. Furthermore, many of the chemical drugs cause harmful side effects in the animals being treated, and as larger doses become required due to the build up of resistance, the side effects become even greater. Moreover, a number of drugs only treat symptoms of a parasitic disease but are unable to prevent infection by the parasitic helminth.

An alternative method to prevent parasitic helminth infection includes administering a vaccine against a parasitic helminth. Although many investigators have tried to develop vaccines based on specific antigens, it is well understood that the ability of an antigen to stimulate antibody production does not necessarily correlate with the ability of the antigen to stimulate an immune response capable of protecting an animal from infection, particularly in the case of parasitic helminths. Although a number of prominent antigens have been identified in several parasitic helminths, there is yet to be a commercially available vaccine developed for any parasitic helminth.

As an example of the complexity of parasitic helminths, the life cycle of *D. immitis*, the helminth that causes heartworm disease, includes a variety of life forms, each of which presents different targets, and challenges, for immunization. In a mosquito, *D. immitis* microfilariae go through two larval stages (L1 and L2) and become mature third stage larvae (L3), which can then be transmitted back to the dog when the mosquito takes a blood meal. In a dog, the L3 molt to the fourth larval stage (L4), and subsequently to the fifth stage, or immature adults. The immature adults migrate to the heart and pulmonary arteries, where they mature to adult heartworms. Adult heartworms are quite large and preferentially inhabit the heart and pulmonary arteries of an animal. Sexually mature adults, after mating, produce microfilariae which traverse capillary beds and circulate in the vascular system of the dog.

In particular, heartworm disease is a major problem in dogs, which typically do not develop immunity, even upon infection (i.e., dogs can become reinfected even after being cured by chemotherapy). In addition, heartworm disease is becoming increasingly widespread in other companion animals, such as cats and ferrets. *D. immitis* has also been reported to infect humans. There remains a need to identify an efficacious composition that protects animals and humans against diseases caused by parasitic helminths, such as

heartworm disease. Preferably, such a composition also protects animals from infection by such helminths.

The parasitic helminth cuticle is a complex extracellular structure which is secreted by an underlying syncytium of hypodermal cells. Recent studies have demonstrated that the cuticle of parasitic helminths is a dynamic structure with important absorptive, secretory, and enzymatic activities, and not merely an inert protective covering as was once believed. See, for example, Lustigman, S. 1993, *Parasitology Today*, 9:8, 294–297. In addition, immunological studies have shown the central importance of cuticular antigens as targets for protective immune responses to parasitic helminths.

Asparaginase amidohydrolases catalyze the hydrolysis of asparagine to aspartic acid and ammonia. See, for example, Moola et al., 1994, *Biochem. J.* 302, 921–927. Studies in systems other than the parasitic helminth indicate that asparaginase is essential for effective hydrolysis of exogenous asparagine and uptake of aspartic acid which cannot otherwise be transported across cell membrane. In yeast, studies have demonstrated that L-asparaginase activity increases in exponentially growing cultures and then decreases as the cells enter the stationary phase. Kim, K. W. and Roon, R. J., 1983, *Biochemistry* 22, 2704–2707. Yeast asparaginase is a highly active cell wall mannan protein and is localized external to the cell membrane and is highly effective in the hydrolysis of exogenous asparagine. *Tetrahymena pyriformis*, a protozoan, cannot transport aspartic acid across its membrane, and L-asparaginase has been shown to be an essential enzyme for aspartic acid-uptake in this species. Tsavdaridis et al., 1991, *Biochemistry International* 24:2, 281–290.

Administration of L-asparaginase in experimental animals and humans leads to regression of certain lymphomas and leukemias, although the exact mechanism by which L-asparaginase kills tumor cells is not clear. See, for example, Moola et al., 1994, *Biochem. J.* 302, 921–927.

SUMMARY OF THE INVENTION

The present invention relates to a novel product and process to protect animals against parasitic helminth infection (e.g., to prevent and/or treat such an infection). The present invention provides parasitic helminth asparaginase proteins and mimetopes thereof; parasitic helminth asparaginase nucleic acid molecules, including those that encode such proteins; antibodies raised against such asparaginase proteins (anti-parasitic helminth asparaginase antibodies); and compounds that inhibit asparaginase activity (i.e., inhibitory compounds or inhibitors).

The present invention also includes methods to obtain parasitic helminth asparaginase proteins, nucleic acid molecules, antibodies and inhibitory compounds. Also included in the present invention are therapeutic compositions comprising such proteins, nucleic acid molecules, antibodies, and inhibitory compounds, as well as use of such therapeutic compositions to protect animals from diseases caused by parasitic helminths.

One embodiment of the present invention is an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a *Dirofilaria immitis* (*D. immitis*) asparaginase gene. Such nucleic acid molecules are referred to as asparaginase nucleic acid molecules. A preferred isolated nucleic acid molecule of this embodiment includes a *D. immitis* asparaginase nucleic acid molecule. A *D. immitis* asparaginase nucleic acid molecule preferably includes nucleic acid sequence SEQ ID NO:1, SEQ ID

NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:25, or SEQ ID NO:26, or allelic variants of any of these sequences.

Another embodiment of the present invention is an isolated nucleic acid molecule that includes a parasitic helminth asparaginase nucleic acid molecule. A preferred parasitic helminth asparaginase nucleic acid molecule of the present invention preferably includes nucleic acid sequence SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:25, or SEQ ID NO:26, or allelic variants of any of these sequences.

The present invention also relates to recombinant molecules, recombinant viruses and recombinant cells that include an isolated asparaginase nucleic acid molecule of the present invention. Also included are methods to produce such nucleic acid molecules, recombinant molecules, recombinant viruses and recombinant cells.

Another embodiment of the present invention includes a non-native parasitic helminth asparaginase protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with a parasitic helminth asparaginase gene. A preferred parasitic helminth protein is capable of eliciting an immune response when administered to an animal and/or of having parasitic helminth asparaginase activity. A preferred parasitic helminth asparaginase protein is encoded by a nucleic acid molecule that hybridizes under stringent conditions with a nucleic acid molecule including either SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:25, or allelic variants of any of these sequences.

Another embodiment of the present invention includes a parasitic helminth asparaginase protein. A preferred asparaginase protein includes a *D. immitis* asparaginase protein. A preferred *D. immitis* asparaginase protein comprises amino acid sequence SEQ ID NO:2, SEQ ID NO:7, or SEQ ID NO:12.

The present invention also relates to: mimetopes of parasitic helminth asparaginase proteins; isolated antibodies that selectively bind to parasitic helminth asparaginase proteins or mimetopes thereof; and inhibitors of parasitic helminth asparaginase proteins or mimetopes thereof. Also included are methods, including recombinant methods, to produce proteins, mimetopes, antibodies, and inhibitors of the present invention.

Another embodiment of the present invention is a method to identify a compound capable of inhibiting parasitic helminth asparaginase activity, comprising the steps of: (a) contacting a parasitic helminth asparaginase protein with a putative inhibitory compound under conditions in which, in the absence of the compound, the protein has asparaginase activity; and (b) determining if the putative inhibitory compound inhibits the asparaginase activity. Also included in the present invention is a test kit to identify a compound capable of inhibiting parasitic helminth asparaginase activity. Such a test kit includes a parasitic helminth asparaginase protein having asparaginase activity and a means for determining the extent of inhibition of the asparaginase activity in the presence of a putative inhibitory compound.

Yet another embodiment of the present invention is a therapeutic composition that is capable of protecting an animal from disease caused by a parasitic helminth. Such a therapeutic composition includes one or more of the following protective compounds: an isolated parasitic helminth asparaginase protein or a mimetope thereof; an isolated

nucleic acid molecule that hybridizes under stringent hybridization conditions with a *Dirofilaria immitis* asparaginase gene; an isolated antibody that selectively binds to a parasitic helminth asparaginase protein; or an inhibitor of asparaginase protein activity identified by its ability to inhibit parasitic helminth asparaginase activity. A preferred therapeutic composition of the present invention also includes an excipient, an adjuvant, or a carrier. Preferred asparaginase nucleic acid molecule therapeutic compositions of the present invention include genetic vaccines, recombinant virus vaccines, and recombinant cell vaccines. Also included in the present invention is a method to protect an animal from disease caused by a parasitic helminth, comprising the step of administering to the animal a therapeutic composition of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for isolated parasitic helminth asparaginase proteins, isolated parasitic helminth asparaginase nucleic acid molecules, isolated antibodies directed against parasitic helminth asparaginase proteins, and other inhibitors of parasitic helminth asparaginase activity. As used herein, the terms isolated parasitic helminth asparaginase proteins, and isolated parasitic helminth asparaginase nucleic acid molecules refers to asparaginase proteins and asparaginase nucleic acid molecules derived from a parasitic helminth and which can be obtained from their natural source, or can be produced using, for example, recombinant nucleic acid technology or chemical synthesis. Also included in the present invention is the use of these proteins, nucleic acid molecules, antibodies and other inhibitors as therapeutic compositions to protect animals from parasitic helminth diseases as well as in other applications, such as those disclosed below.

The present invention is based on the surprising discovery of asparaginase in parasitic helminth cuticle. Parasitic helminth asparaginase proteins and nucleic acid molecules of the present invention have utility because they represent novel targets for anti-parasite vaccines and drugs. The products and processes of the present invention are advantageous because they enable the inhibition of parasite physiological functions that depend on asparaginase activity.

To the inventors' knowledge, the present invention is the first disclosure of a protein or nucleic acid molecule exhibiting significant similarity to known asparaginases or asparaginase genes, respectively, being isolated from a parasitic helminth.

One embodiment of the present invention is an isolated protein comprising a parasitic helminth asparaginase protein. It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, a protein refers to one or more proteins or at least one protein. As such, the terms "a" (or "an"), "one or more" and "at least one" can be used interchangeably herein. It is also to be noted that the terms "comprising", "including", and "having" can be used interchangeably. Furthermore, a compound "selected from the group consisting of" refers to one or more of the compounds in the list that follows, including mixtures (i.e., combinations) of two or more of the compounds. According to the present invention, an isolated, or biologically pure, protein, is a protein that has been removed from its natural milieu. The terms "isolated" and "biologically pure" do not necessarily reflect the extent to which the protein has been purified. An isolated protein of the present invention can be obtained from its natural source, can be produced using

recombinant DNA technology or can be produced by chemical synthesis. When an isolated protein of the present invention is produced using recombinant DNA technology or produced by chemical synthesis, the protein is referred to herein as either an isolated protein or as a non-native protein.

As used herein, an isolated parasitic helminth asparaginase protein can be a full-length protein or any homolog of such a protein. An isolated protein of the present invention, including a homolog, can be identified in a straight-forward manner by the protein's ability to elicit an immune response against a parasitic helminth asparaginase protein or to catalyze the cleavage of asparagine to aspartic acid and ammonia. Examples of parasitic helminth asparaginase homologs include parasitic helminth asparaginase proteins in which amino acids have been deleted (e.g., a truncated version of the protein, such as a peptide), inserted, inverted, substituted and/or derivatized (e.g., by glycosylation, phosphorylation, acetylation, myristoylation, prenylation, palmitoylation, amidation, or addition of glycerophosphatidyl inositol) so that the homolog includes at least one epitope capable of eliciting an immune response against a parasitic helminth asparaginase protein. That is, when the homolog is administered to an animal as an immunogen, using techniques known to those skilled in the art, the animal will produce an immune response against at least one epitope of a natural parasitic helminth asparaginase protein. As used herein, the term "epitope" refers to the smallest portion of a protein or other antigen capable of selectively binding to the antigen binding site of an antibody or a T-cell receptor. It is well accepted by those skilled in the art that the minimal size of a protein epitope is about four amino acids. The ability of a protein to effect an immune response can be measured using techniques known to those skilled in the art.

Parasitic helminth asparaginase protein homologs can be the result of natural allelic variation or natural mutation. Parasitic helminth asparaginase protein homologs of the present invention can also be produced using techniques known in the art including, but not limited to, direct modifications to the protein or modifications to the gene encoding the protein using, for example, classic or recombinant DNA techniques to effect random or targeted mutagenesis.

An asparaginase protein of the present invention is encoded by a parasitic helminth asparaginase nucleic acid molecule. As used herein, a parasitic helminth asparaginase nucleic acid molecule includes a nucleic acid sequence related to a natural parasitic helminth asparaginase gene, and preferably, to a *D. immitis* asparaginase gene. As used herein, a parasitic helminth asparaginase gene includes all regions that control production of the parasitic helminth asparaginase protein encoded by the gene (such as, but not limited to, transcription, translation or post-translation control regions) as well as the coding region itself, and any introns or non-translated coding regions. As used herein, a gene that "includes" or "comprises" a nucleic acid sequence may include that sequence in one contiguous array, or may include that sequence as fragmented exons. As used herein, the term "coding region" refers to a continuous linear array of nucleotides that translates into a protein. A full-length coding region is that coding region which is translated into a full-length, i.e., a complete, protein as would be initially translated in its natural milieu, prior to any post-translational modifications.

In one embodiment, a parasitic helminth asparaginase gene of the present invention includes the nucleic acid molecule nDiAsp₁₇₅₃, which is herein represented by the nucleic acid sequence SEQ ID NO:1 (the coding strand), as well as the complement of SEQ ID NO:1. The production of

nDiAsp₁₇₅₃ is disclosed in the Examples. The complement of SEQ ID NO:1 (represented herein by SEQ ID NO:3) refers to the nucleic acid sequence of the strand complementary to the strand having SEQ ID NO:1, which can easily be determined by those skilled in the art. Likewise, a nucleic acid sequence complement of any nucleic acid sequence of the present invention refers to the nucleic acid sequence of the nucleic acid strand that is complementary to (i.e., can form a double helix with) the strand for which the sequence is cited.

In another embodiment, a parasitic helminth asparaginase gene of the present invention includes the nucleic acid sequence SEQ ID NO:6, as well as the complement of SEQ ID NO:6. Nucleic acid sequence SEQ ID NO:6 represents the nucleic acid sequence of the coding strand of the nucleic acid molecule denoted herein as nDiAsp₄₃₉, the production of which is disclosed in the Examples. The complement of SEQ ID NO:6 (represented herein by SEQ ID NO:8) refers to the nucleic acid sequence of the strand complementary to the strand having SEQ ID NO:6.

In another embodiment, a parasitic helminth asparaginase gene of the present invention includes the nucleic acid sequence SEQ ID NO:11, as well as the complement of SEQ ID NO:11. Nucleic acid sequence SEQ ID NO:11 represents the nucleic acid sequence of the coding strand of the nucleic acid molecule denoted herein as nDiAsp₁₇₇₀, the production of which is disclosed in the Examples. The complement of SEQ ID NO:11 (represented herein by SEQ ID NO:13) refers to the nucleic acid sequence of the strand complementary to the strand having SEQ ID NO:11.

In another embodiment, a parasitic helminth asparaginase gene of the present invention includes the nucleic acid sequence SEQ ID NO:25, as well as the complement of SEQ ID NO:25. Nucleic acid sequence SEQ ID NO:25 represents the nucleic acid sequence of the coding strand of the nucleic acid molecule denoted herein as nDiAsp₂₀₇₃, the production of which is disclosed in the Examples. Nucleic acid molecule nDiAsp₂₀₇₃ includes the sequence of the isolated coding strand of the apparent full length cDNA encoding a parasitic helminth asparaginase protein expressed in *D. immitis*. The complement of SEQ ID NO:25 (represented herein by SEQ ID NO:26) refers to the nucleic acid sequence of the strand complementary to the strand having SEQ ID NO:25.

In another embodiment, a parasitic helminth asparaginase gene can be an allelic variant that includes a similar, but not identical, sequence to SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:25, or SEQ ID NO:26, or any other nucleic acid sequence cited herein. For example, an allelic variant of a parasitic helminth asparaginase gene including SEQ ID NO:25 and SEQ ID NO:26 is a gene that occurs at essentially the same locus (or loci) in the genome as the gene including SEQ ID NO:25 and SEQ ID NO:26, but which, due to natural variations caused by, for example, mutation or recombination, has a similar but not identical sequence. Because natural selection typically selects against alterations that affect function, an allelic variant usually encodes a protein having a similar activity or function to that of the protein encoded by the gene to which it is being compared. An allelic variant of a gene or nucleic acid molecule can also comprise alterations in the 5' or 3' untranslated regions of the gene (e.g., in regulatory control regions), or can involve alternative splicing of a nascent transcript, thereby bringing alternative exons into juxtaposition. Allelic variants are well known to those skilled in the art and would be expected to

be found naturally occurring within parasitic helminths because the helminth genome is diploid, and sexual reproduction will result in the reassortment of alleles.

In one embodiment of the present invention, isolated asparaginase proteins are encoded by nucleic acid molecules that hybridize under stringent hybridization conditions to a gene encoding a parasitic helminth asparaginase protein (i.e., to a *D. immitis* asparaginase gene). The minimal size of an asparaginase protein of the present invention is a size sufficient to be encoded by a nucleic acid molecule capable of forming a stable hybrid (i.e., hybridize under stringent hybridization conditions) with the complementary sequence of a nucleic acid molecule encoding the corresponding natural protein. As used herein, "stringent hybridization conditions" refer to those experimental conditions under which nucleic acid molecules having similar nucleic acid sequences will anneal to each other. Stringent hybridization conditions, as defined herein, permit the hybridization of nucleic acid molecules having at least about 70% nucleic acid sequence identity with the nucleic acid molecule being used as a probe in the hybridization reaction, i.e., permit the hybridization of a nucleic acid molecule to a probe having up to about 30% base-pair mismatch. Formulae to calculate the appropriate hybridization and wash conditions to achieve hybridization permitting 30% or less mismatch between two nucleic acid molecules are disclosed, for example, in Meinkoth et al, 1984, *Anal. Biochem* 138, 267-284; Meinkoth et al, *ibid*, is incorporated by reference herein in its entirety. The size of a nucleic acid molecule encoding such a protein homolog is dependent on the nucleic acid composition and the percent homology between the nucleic acid molecule and complementary sequence. It should also be noted that the extent of homology required to form a stable hybrid can vary depending on whether the homologous sequences are interspersed throughout a given nucleic acid molecule or are clustered (i.e., localized) in distinct regions on a given nucleic acid molecule. The minimal size of such a nucleic acid molecule is typically at least about 12 to about 15 nucleotides in length if the nucleic acid molecule is GC-rich and at least about 15 to about 17 bases in length if it is AT-rich. As such, the minimal size of a nucleic acid molecule used to encode an asparaginase protein homolog of the present invention is from about 12 to about 18 nucleotides in length. Thus, the minimal size of an asparaginase protein homolog of the present invention is from about 4 to about 6 amino acids in length. There is no limit, other than a practical limit, on the maximal size of such a nucleic acid molecule in that the nucleic acid molecule can include a portion of a gene, an entire gene, or multiple genes. The preferred size of a protein encoded by a nucleic acid molecule of the present invention depends on whether a full-length, fusion, multivalent, or functional portion of such a protein is desired.

One embodiment of the present invention includes a parasitic helminth protein having asparaginase enzyme activity. Such an asparaginase protein preferably includes the ability to catalyze the cleavage of asparagine to aspartic acid and ammonia.

A preferred parasitic helminth asparaginase protein of the present invention is a compound that when administered to an animal in an effective manner, is capable of protecting that animal from disease caused by a parasitic helminth. In accordance with the present invention, the ability of an asparaginase protein of the present invention to protect an animal from disease by a parasitic helminth refers to the ability of that protein to, for example, treat, ameliorate or prevent disease caused by parasitic helminths. In one

embodiment, a parasitic helminth asparaginase protein of the present invention can elicit an immune response (including a humoral and/or cellular immune response) against a parasitic helminth.

Suitable parasites to target include any parasite that is essentially incapable of causing disease in an animal administered a parasitic helminth asparaginase protein of the present invention. Accordingly, a parasite to target includes any parasite that produces a protein having one or more epitopes that can be targeted by a humoral or cellular immune response against a parasitic helminth asparaginase protein of the present invention or that can be targeted by a compound that otherwise inhibits parasite asparaginase activity, thereby resulting in the decreased ability of the parasite to cause disease in an animal. Preferred parasites to target include parasitic helminths such as nematodes, cestodes, and trematodes, with nematodes being preferred. Preferred nematodes to target include filariid, ascarid, capillarid, strongylid, strongyloides, trichostrongyle, and trichurid nematodes. Particularly preferred nematodes are those of the genera *Acanthocheilonema*, *Aelurostrongylus*, *Ancylostoma*, *Angiostrongylus*, *Ascaris*, *Brugia*, *Bunostomum*, *Capillaria*, *Chabertia*, *Cooperia*, *Crenosoma*, *Dictyocaulus*, *Dioctophyme*, *Dipetalonema*, *Diphyllobothrium*, *Diplydium*, *Dirofilaria*, *Dracunculus*, *Enterobius*, *Filaroides*, *Haemonchus*, *Lagochilascaris*, *Loa*, *Mansonella*, *Muellerius*, *Nanophyetus*, *Necator*, *Nematodirus*, *Oesophagostomum*, *Onchocerca*, *Opisthorchis*, *Ostertagia*, *Parafilaria*, *Paragonimus*, *Parascaris*, *Physaloptera*, *Protostrongylus*, *Setaria*, *Spirocerca*, *Spirometra*, *Stephanofilaria*, *Strongyloides*, *Strongylus*, *Thelazia*, *Toxascaris*, *Toxocara*, *Trichinella*, *Trichostrongylus*, *Trichuris*, *Uncinaria*, and *Wuchereria*. Preferred filariid nematodes include *Dirofilaria*, *Onchocerca*, *Acanthocheilonema*, *Brugia*, *Dipetalonema*, *Loa*, *Parafilaria*, *Setaria*, *Stephanofilaria* and *Wuchereria* filariid nematodes, with *D. immitis* being even more preferred.

The present invention also includes mimetopes of parasitic helminth asparaginase proteins of the present invention. As used herein, a mimetope of a parasitic helminth asparaginase protein of the present invention refers to any compound that is able to mimic the activity of a parasitic helminth asparaginase protein (e.g., has the ability to elicit an immune response against a parasitic helminth asparaginase protein of the present invention or ability to inhibit parasitic helminth asparaginase activity). The ability to mimic the activity of a parasitic helminth asparaginase protein is likely to be the result of a structural similarity between the parasitic helminth asparaginase protein and the mimetope. It is to be noted, however, that the mimetope need not have a structure similar to a parasitic helminth asparaginase protein as long as the mimetope functionally mimics the protein. A mimetope can be, but is not limited to: a peptide that has been modified to decrease its susceptibility to degradation (e.g., as an all-D retro peptide); an anti-idiotypic or catalytic antibody, or a fragment thereof; a non-proteinaceous immunogenic portion of an isolated protein (e.g., a carbohydrate structure); or a synthetic or natural organic molecule, including a nucleic acid. Such a mimetope can be designed using computer-generated structures of proteins of the present invention. A mimetope can also be obtained by generating random samples of molecules, such as oligonucleotides, peptides or other organic molecules, and screening such samples by affinity chromatography techniques using the corresponding binding partner.

In one embodiment, a parasitic helminth asparaginase protein of the present invention is a fusion protein that

includes a parasitic helminth asparaginase protein-containing domain attached to one or more fusion segments. Suitable fusion segments for use with the present invention include, but are not limited to, segments that can: enhance a protein's stability; act as an immunopotentiator to enhance an immune response against a parasitic helminth asparaginase protein; or assist purification of a parasitic helminth asparaginase protein (e.g., by affinity chromatography). A suitable fusion segment can be a domain of any size that has the desired function (e.g., imparts increased stability, imparts increased immunogenicity to a protein, or simplifies purification of a protein). Fusion segments can be joined to the amino or carboxyl termini of a parasitic helminth asparaginase protein-containing domain, and can be susceptible to cleavage in order to enable straight-forward recovery of a parasitic helminth asparaginase protein. A fusion protein is preferably produced by culturing a recombinant cell transformed with a fusion nucleic acid molecule that encodes a protein including a fusion segment attached to either the carboxyl or amino terminal end of an asparaginase protein-containing domain. Preferred fusion segments include a metal binding domain (e.g., a poly-histidine segment); an immunoglobulin binding domain (e.g., Protein A; Protein G; T cell; B cell; Fc receptor or complement protein antibody-binding domains); a sugar binding domain (e.g., a maltose binding domain); and/or a "tag" domain (e.g., at least a portion of β -galactosidase, a strep tag peptide, a T7-tag peptide, a FLAGTM peptide, or other domain that can be purified using compounds that bind to the domain, such as monoclonal antibodies). More preferred fusion segments include metal binding domains, such as a poly-histidine segment; a maltose binding domain; a strep tag peptide, such as that available from Biometra® in Tampa, Fla.; and an S10 peptide. An example of a particularly preferred fusion protein of the present invention is PHIS-PDiAsp₅₉₀, production of which is disclosed herein.

In another embodiment, a parasitic helminth asparaginase protein of the present invention also includes at least one additional protein segment that is capable of protecting an animal from one or more diseases. Such a multivalent protective protein can be produced by culturing a cell transformed with a nucleic acid molecule comprising two or more nucleic acid domains joined together in such a manner that the resulting nucleic acid molecule is expressed as a multivalent protective compound containing at least two protective compounds, or portions thereof, capable of protecting an animal from diseases caused, for example, by at least one infectious agent.

Examples of multivalent protective compounds include, but are not limited to, a parasitic helminth asparaginase protein of the present invention attached to one or more compounds protective against one or more other infectious agents, particularly an agent that infects humans, cats, dogs, ferrets, cattle or horses, such as, but not limited to: viruses (e.g., adenoviruses, caliciviruses, coronaviruses, distemper viruses, hepatitis viruses, herpesviruses, immunodeficiency viruses, infectious peritonitis viruses, leukemia viruses, oncogenic viruses, panleukopenia viruses, papilloma viruses, parainfluenza viruses, parvoviruses, rabies viruses, and reoviruses, as well as other cancer-causing or cancer-related viruses); bacteria (e.g., Actinomyces, Bacillus, Bacteroides, Bordetella, Bartonella, Borrelia, Brucella, Campylobacter, Capnocytophaga, Clostridium, Corynebacterium, Coxiella, Dermatophilus, Enterococcus, Ehrlichia, Escherichia, Francisella, Fusobacterium, Haemobartonella, Helicobacter, Klebsiella, L-form bacteria, Leptospira, Listeria, Mycobacteria, Mycoplasma,

Neorickettsia, Nocardia, Pasteurella, Peptococcus, Peptostreptococcus, Proteus, Pseudomonas, Rickettsia, Rochalimaea, Salmonella, Shigella, Staphylococcus, Streptococcus, and Yersinia; fungi and fungal-related microorganisms (e.g., Absidia, Acremonium, Alternaria, Aspergillus, Basidiobolus, Bipolaris, Blastomyces, Candida, Chlamydia, Coccidioides, Conidiobolus, Cryptococcus, Curvalaria, Epidermophyton, Exophiala, Geotrichum, Histoplasma, Madurella, Malassezia, Microsporum, Moniliella, Mortierella, Mucor, Paecilomyces, Penicillium, Phialemonium, Phialophora, Prototheca, Pseudallescheria, Pseudomicrodochium, Pythium, Rhinosporidium, Rhizopus, Scolecobasidium, Sporothrix, Stemphylium, Trichophyton, Trichosporon, and Xylohypha; and other parasites (e.g., Babesia, Balantidium, Besnoitia, Cryptosporidium, Eimeria, Encephalitozoon, Entamoeba, Giardia, Hammondia, Hepatozoon, Isospora, Leishmania, Microsporidia, Neospora, Nosema, Pentatrichomonas, Plasmodium, Pneumocystis, Sarcocystis, Schistosoma, Theileria, Toxoplasma, and Trypanosoma, as well as helminth parasites, such as those disclosed herein). In one embodiment, a parasitic helminth asparaginase protein of the present invention is attached to one or more additional compounds protective against heartworm disease. In another embodiment, one or more protective compounds, such as those listed above, can be included in a multivalent vaccine comprising a parasitic helminth asparaginase protein of the present invention and one or more other protective molecules as separate compounds.

In one embodiment, a preferred isolated asparaginase protein of the present invention is a protein encoded by a nucleic acid molecule comprising at least a portion of nDiAsp₁₇₅₃, nDiAsp₁₅₁₈, nDiAsp₄₃₉, nDiAsp₃₆₉, nDiAsp₁₇₇₀, or nDiAsp₂₀₇₃, or by an allelic variant of any of these nucleic acid molecules. Also preferred is an isolated asparaginase protein encoded by a nucleic acid molecule having the nucleic acid sequence SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:11 or SEQ ID NO:25; or by an allelic variant of a nucleic acid molecule having any of these sequences.

Translation of SEQ ID NO:1, the coding strand of nucleic acid molecule nDiAsp₁₇₅₃, yields a partial length parasitic helminth asparaginase protein of 506 amino acids, referred to herein as PDiAsp₅₀₆, the amino acid sequence of which is represented by SEQ ID NO:2. The open reading frame spans from nucleotide 1 through nucleotide 1518 of SEQ ID NO:1 and a termination (stop) codon spans from nucleotide 1519 through nucleotide 1521 of SEQ ID NO:1. The coding region encoding PDiAsp₅₀₆, not including the stop codon, is represented by nucleic acid molecule nDiAsp₁₅₁₈, having the nucleic acid sequence represented by SEQ ID NO:4 (the coding strand) and SEQ ID NO:5 (the complementary strand). The deduced amino acid sequence SEQ ID NO:2 encodes a protein having a molecular weight of 56.4 kilodaltons (kD) and an estimated pI of about 6.44. The 3' end of the non-coding region of SEQ ID NO:1 has a polyadenylation signal, AATAAA, that spans from nucleotide 1535 to nucleotide 1540, followed by a 20 nucleotide poly-A tail.

Translation of SEQ ID NO:6, the coding strand of nucleic acid molecule nDiAsp₄₃₉, yields a partial length parasitic helminth asparaginase protein of 123 amino acids, referred to herein as PDiAsp₁₂₃, the amino acid sequence of which is represented by SEQ ID NO:7, assuming an open reading frame that spans from nucleotide 69 through nucleotide 71 of SEQ ID NO:6. The coding region encoding PDiAsp₁₂₃ is represented by nucleic acid molecule nDiAsp₃₆₉, having the nucleic acid sequence represented by SEQ ID NO:9 (the

coding strand) and SEQ ID NO:10 (the complementary strand). The deduced amino acid sequence SEQ ID NO:7 encodes a protein having a molecular weight of about 14.3 kilodaltons (kD) and an estimated pI of about 4.16.

Translation of SEQ ID NO:11, the coding strand of nucleic acid molecule nDiAsp₁₇₇₀, yields an apparent full length parasitic helminth asparaginase protein of 590 amino acids, referred to herein as PDiAsp₅₉₀, the amino acid sequence of which is represented by SEQ ID NO:12, assuming an open reading frame that spans from nucleotide 1 through nucleotide 1770 of SEQ ID NO:11. SEQ ID NO:12 encodes a protein having a molecular weight of about 66.2 kilodaltons (kD) and an estimated pI of about 5.96.

One embodiment of the present invention includes a non-native parasitic helminth asparaginase protein encoded by a nucleic acid molecule that hybridizes under stringent hybridization conditions with a parasitic helminth asparaginase gene. A preferred parasitic helminth protein is capable of eliciting an immune response when administered to an animal and/or of having parasitic helminth asparaginase activity. A preferred parasitic helminth asparaginase protein is encoded by a nucleic acid molecule that hybridizes under stringent conditions with a nucleic acid molecule including either SEQ ID NO:1, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:9, SEQ ID NO:11, or SEQ ID NO:25.

A preferred asparaginase protein of the present invention comprises a protein that is that is at least about 60%, more preferably at least about 65%, more preferably at least about 70%, more preferably at least about 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90%, and even more preferably at least about 95% identical to identical to PDiAsp₅₀₆, PDiAsp₁₂₃ or PDiAsp₅₉₀. More preferred is an asparaginase protein comprising PDiAsp₅₀₆, PDiAsp₁₂₃, or PDiAsp₅₉₀, or a protein encoded by an allelic variant of a nucleic acid molecule encoding a protein comprising PDiAsp₅₀₆, PDiAsp₁₂₃, or PDiAsp₅₉₀.

Also preferred is an asparaginase protein comprising an amino acid sequence that is at least about 60%, more preferably at least about 65%, more preferably at least about 70%, more preferably at least about 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90%, and even more preferably at least about 95%, identical to amino acid sequence SEQ ID NO:2, SEQ ID NO:7, or SEQ ID NO:12. Even more preferred is an amino acid sequence having the sequence represented by SEQ ID NO:2, SEQ ID NO:7, or SEQ ID NO:12, or an allelic variant of an amino acid sequence having the sequence represented by SEQ ID NO:2, SEQ ID NO:7, or SEQ ID NO:12.

A particularly preferred parasitic helminth asparaginase protein of the present invention comprises amino acid sequence SEQ ID NO:12, including, but not limited to, an asparaginase protein consisting of amino acid sequence SEQ ID NO:12, a fusion protein or a multivalent protein; or a protein encoded by an allelic variant of a nucleic acid molecule encoding a protein having amino acid sequence SEQ ID NO:12.

Another embodiment of the present invention is an isolated nucleic acid molecule comprising a parasitic helminth asparaginase nucleic acid molecule. The identifying characteristics of such a nucleic acid molecule are heretofore described. A nucleic acid molecule of the present invention can include an isolated natural parasitic helminth asparaginase gene or a homolog thereof, the latter of which is described in more detail below. A nucleic acid molecule of

the present invention can include one or more regulatory regions, a full-length or a partial coding region, or a combination thereof. The minimal size of a nucleic acid molecule of the present invention is a size sufficient to allow the formation of a stable hybrid (i.e., hybridization under stringent hybridization conditions) with the complementary sequence of another nucleic acid molecule. Accordingly, the minimal size of an asparaginase nucleic acid molecule of the present invention is from about 12 to about 18 nucleotides in length. A preferred asparaginase nucleic acid molecule includes a parasitic helminth asparaginase nucleic acid molecule.

In accordance with the present invention, an isolated nucleic acid molecule is a nucleic acid molecule that has been removed from its natural milieu (i.e., that has been subject to human manipulation) and can include DNA, RNA, or derivatives of either DNA or RNA. As such, "isolated" does not reflect the extent to which the nucleic acid molecule has been purified. An isolated parasitic helminth asparaginase nucleic acid molecule of the present invention can be isolated from its natural source or produced using recombinant DNA technology (e.g., polymerase chain reaction (PCR) amplification or cloning) or chemical synthesis. Isolated parasitic helminth asparaginase nucleic acid molecules can include, for example, natural allelic variants and nucleic acid molecules modified by nucleotide insertions, deletions, substitutions, or inversions in a manner such that the modifications do not substantially interfere with the nucleic acid molecule's ability to encode an asparaginase protein of the present invention.

A parasitic helminth asparaginase nucleic acid molecule homolog can be produced using a number of methods known to those skilled in the art. See, for example, Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Labs Press; Sambrook et al., *ibid.*, is incorporated by reference herein in its entirety. For example, a nucleic acid molecule can be modified using a variety of techniques including, but not limited to, classic mutagenesis and recombinant DNA techniques such as site-directed mutagenesis, chemical treatment, restriction enzyme cleavage, ligation of nucleic acid fragments, PCR amplification, synthesis of oligonucleotide mixtures and ligation of mixture groups to "build" a mixture of nucleic acid molecules, and combinations thereof. A nucleic acid molecule homolog can be selected by hybridization with a parasitic helminth asparaginase nucleic acid molecule or by screening the function of a protein encoded by the nucleic acid molecule (e.g., ability to elicit an immune response against at least one epitope of a parasitic helminth asparaginase protein, or the ability to demonstrate asparaginase activity).

An isolated nucleic acid molecule of the present invention can include a nucleic acid sequence that encodes a parasitic helminth asparaginase protein of the present invention, examples of such proteins being disclosed herein. Although the phrase "nucleic acid molecule" primarily refers to the physical nucleic acid molecule and the phrase "nucleic acid sequence" primarily refers to the sequence of nucleotides on the nucleic acid molecule, the two phrases can be used interchangeably, especially with respect to a nucleic acid molecule, or a nucleic acid sequence, being capable of encoding a parasitic helminth asparaginase protein.

A preferred nucleic acid molecule of the present invention, when administered to an animal, is capable of protecting that animal from disease caused by a parasitic helminth. As will be disclosed in more detail below, such a nucleic acid molecule can be, or can encode, an antisense

RNA, a molecule capable of triple helix formation, a ribozyme, or other nucleic acid-based drug compound. In additional embodiments, a nucleic acid molecule of the present invention can encode a protective protein (e.g., an asparaginase protein of the present invention), the nucleic acid molecule being delivered to the animal, for example, by direct injection (i.e., as a genetic vaccine) or in a vehicle such as a recombinant virus vaccine or a recombinant cell vaccine.

One embodiment of the present invention is an isolated nucleic acid molecule that hybridizes under stringent hybridization conditions with a parasitic helminth asparaginase gene. Preferred parasitic helminth asparaginase genes of the present invention are asparaginase genes from *Dirofilaria immitis*. Such nucleic acid molecules are referred to as parasitic helminth asparaginase nucleic acid molecules. A parasitic helminth asparaginase gene preferably includes at least one of the following nucleic acid sequences: SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:25, or SEQ ID NO:26.

In another embodiment, a parasitic helminth asparaginase nucleic acid molecule of the present invention includes a nucleic acid molecule that is at least about 70%, more preferably at least about 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90%, and even more preferably at least about 95% identical to nucleic acid molecule nDiAsp₁₇₅₃, nDiAsp₁₅₁₈, nDiAsp₄₃₉, nDiAsp₃₆₉, nDiAsp₁₇₇₀, or nDiAsp₂₀₇₃, or an allelic variant of any of these nucleic acid molecules. Also preferred is a parasitic helminth asparaginase nucleic acid molecule comprising a nucleic acid sequence that is at least about 70%, more preferably at least about 75%, more preferably at least about 80%, more preferably at least about 85%, more preferably at least about 90%, and even more preferably at least about 95% identical to nucleic acid sequence SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:25, or SEQ ID NO:26; or an allelic variant of a nucleic acid molecule having any of these sequences.

Particularly preferred is an asparaginase nucleic acid molecule comprising all or part of nucleic acid molecule nDiAsp₁₇₅₃, nDiAsp₁₅₁₈, nDiAsp₄₃₉, nDiAsp₃₆₉, nDiAsp₁₇₇₀, or nDiAsp₂₀₇₃, or an allelic variant of any these nucleic acid molecules. Also particularly preferred is a nucleic acid molecule that includes at least a portion of nucleic acid sequence SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:25, or SEQ ID NO:26, or an allelic variant of a nucleic acid molecule having any of these nucleic acid sequences. Such a nucleic acid molecule can include nucleotides in addition to those included in the SEQ ID NOs, such as, but not limited to, nucleotides comprising a full-length gene, or nucleotides comprising a nucleic acid molecule encoding a fusion protein or a nucleic acid molecule encoding a multivalent protective compound.

The present invention also includes a nucleic acid molecule encoding a protein having at least a portion of SEQ ID NO:12, or an allelic variant of a nucleic acid molecule encoding a protein having at least a portion of SEQ ID NO:12. The present invention further includes a nucleic acid molecule that has been modified to accommodate codon usage properties of a cell in which such a nucleic acid molecule is to be expressed.

Knowing the nucleic acid sequences of certain parasitic helminth asparaginase nucleic acid molecules of the present invention allows one skilled in the art to, for example, (a) make copies of those nucleic acid molecules, (b) obtain nucleic acid molecules including at least a portion of such nucleic acid molecules (e.g., nucleic acid molecules including full-length genes, full-length coding regions, regulatory control sequences, truncated coding regions), and (c) obtain other parasitic helminth asparaginase nucleic acid molecules. Such nucleic acid molecules can be obtained in a variety of ways including screening appropriate expression libraries with antibodies of the present invention; traditional cloning techniques using oligonucleotide probes of the present invention to screen appropriate libraries; and PCR amplification of appropriate libraries or DNA using oligonucleotide primers of the present invention. Preferred libraries to screen or from which to amplify nucleic acid molecules include *Dirofilaria* L3, L4 or adult cDNA libraries as well as genomic DNA libraries. Similarly, preferred DNA sources from which to amplify nucleic acid molecules include *Dirofilaria* L3, L4 or adult first-strand cDNA syntheses and genomic DNA. Techniques to clone and amplify genes are disclosed, for example, in Sambrook et al., *ibid*.

The present invention also includes a nucleic acid molecule that is an oligonucleotide capable of hybridizing, under stringent hybridization conditions, with complementary regions of other, preferably longer, nucleic acid molecules of the present invention such as those comprising parasitic helminth asparaginase nucleic acid molecules; or with complementary regions of other parasitic helminth asparaginase nucleic acid molecules. An oligonucleotide of the present invention can be RNA, DNA, or derivatives of either. The minimum size of such an oligonucleotide is the size required for formation of a stable hybrid between the oligonucleotide and a complementary sequence on another nucleic acid molecule. A preferred oligonucleotide of the present invention has a maximum size of about 100 nucleotides. The present invention includes oligonucleotides that can be used as, for example, probes to identify nucleic acid molecules, primers to produce nucleic acid molecules, or therapeutic reagents to inhibit parasitic helminth asparaginase protein production or activity (e.g., as antisense-, triplex formation-, ribozyme- and/or RNA drug-based reagents). The present invention also includes the use of such oligonucleotides to protect animals from disease using one or more of such technologies. Appropriate oligonucleotide-containing therapeutic compositions can be administered to an animal using techniques known to those skilled in the art.

Another embodiment of the present invention includes a recombinant vector, which includes at least one isolated nucleic acid molecule of the present invention inserted into any vector capable of delivering the nucleic acid molecule into a host cell. Such a vector contains heterologous nucleic acid sequences, that is, nucleic acid sequences that are not naturally found adjacent to nucleic acid molecules of the present invention, and that preferably are derived from a species other than the species from which the nucleic acid molecule(s) are derived. The vector can be either RNA or DNA, either prokaryotic or eukaryotic, and typically is a virus or a plasmid. Recombinant vectors can be used to clone, sequence, or otherwise manipulate a parasitic helminth asparaginase nucleic acid molecule of the present invention.

One type of recombinant vector, referred to herein as a recombinant molecule, comprises a nucleic acid molecule of the present invention operatively linked to an expression

vector. The phrase "operatively linked" refers to insertion of a nucleic acid molecule into an expression vector in a manner such that the molecule is able to be expressed when transformed into a host cell. As used herein, an expression vector is a DNA or RNA vector that is capable of transforming a host cell and of effecting expression of a specified nucleic acid molecule. Preferably, the expression vector is also capable of replicating within the host cell. An expression vector can be either prokaryotic or eukaryotic, and is typically a virus or a plasmid. An expression vector of the present invention includes any vector that functions (i.e., directs gene expression) in a recombinant cell of the present invention, including in a bacterial, fungal, parasite, insect, other animal, or plant cell. A preferred expression vector of the present invention can direct gene expression in a bacterial, yeast, helminth or other parasite, insect or mammalian cell, or more preferably in a cell type disclosed herein.

In particular, an expression vector of the present invention contains regulatory sequences such as transcription control sequences, translation control sequences, origins of replication, and other regulatory sequences that are compatible with the recombinant cell and that control the expression of a nucleic acid molecule of the present invention. In particular, a recombinant molecule of the present invention includes transcription control sequences. Transcription control sequences are sequences which control the initiation, elongation, and termination of transcription. Particularly important transcription control sequences are those which control transcription initiation, such as promoter, enhancer, operator and repressor sequences. A suitable transcription control sequence includes any transcription control sequence that can function in at least one of the recombinant cells of the present invention. A variety of such transcription control sequences are known to those skilled in the art. Preferred transcription control sequences include those which function in bacterial, yeast, helminth or other parasite, insect or mammalian cells, such as, but not limited to, *tac*, *lac*, *trp*, *trc*, *oxy-pro*, *omp/lpp*, *rrnB*, bacteriophage lambda (such as lambda *p_L*, and lambda *p_R* and fusions that include such promoters), bacteriophage T7, T7lac, bacteriophage T3, bacteriophage SP6, bacteriophage SP01, metallothionein, alpha-mating factor, *Pichia* alcohol oxidase, alphavirus subgenomic promoters (such as Sindbis virus subgenomic promoters), antibiotic resistance gene, baculovirus, *Heliothis zea* insect virus, vaccinia virus, herpesvirus, raccoon poxvirus, other poxvirus, adenovirus, cytomegalovirus (such as immediate early promoters), picornavirus, simian virus 40, retrovirus, actin, retroviral long terminal repeat, Rous sarcoma virus, heat shock, phosphate or nitrate transcription control sequences; as well as other sequences capable of controlling gene expression in prokaryotic or eukaryotic cells. Additional suitable transcription control sequences include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (e.g., promoters inducible by interferons or interleukins). Transcription control sequences of the present invention can also include naturally occurring transcription control sequences naturally associated with parasitic helminths, such as *D immitis* or *B. malayi*.

Suitable and preferred nucleic acid molecules to include in a recombinant vector of the present invention are as disclosed herein. Preferred nucleic acid molecules to include in a recombinant vector, and particularly in a recombinant molecule, include nDiAsp₁₇₅₃, nDiAsp₁₅₁₈, nDiAsp₄₃₉, nDiAsp₃₆₉, nDiAsp₁₇₇₀, and nDiAsp₂₀₇₃. A particularly preferred recombinant molecule of the present invention is

PHis-DiAsp₁₇₇₀, the production of which is described in the Examples section.

A recombinant molecule of the present invention may also (a) contain a secretory signal (i.e., a signal segment nucleic acid sequence) to enable an expressed asparaginase protein of the present invention to be secreted from the cell that produces the protein or (b) contain a fusion sequence which leads to the expression of a nucleic acid molecule of the present invention as a fusion protein. Examples of suitable signal segments include any signal segment capable of directing the secretion of a protein of the present invention. Preferred signal segments include, but are not limited to, native parasitic helminth signal segments, tissue plasminogen activator (t-PA), interferon, interleukin, growth hormone, histocompatibility and viral envelope glycoprotein signal segments. Suitable fusion segments encoded by fusion segment nucleic acids are disclosed herein. In addition, a nucleic acid molecule of the present invention can be joined to a fusion segment that directs the encoded protein to the proteasome, such as a ubiquitin fusion segment. A eukaryotic recombinant molecule may also include intervening and/or untranslated sequences surrounding and/or within the nucleic acid sequence of the nucleic acid molecule of the present invention.

Another embodiment of the present invention includes a recombinant cell comprising a host cell transformed with one or more recombinant molecules of the present invention. Transformation of a nucleic acid molecule into a cell can be accomplished by any method by which a nucleic acid molecule can be inserted into the cell. Transformation techniques include, but are not limited to, transfection, electroporation, microinjection, lipofection, adsorption, and protoplast fusion. A recombinant cell may remain unicellular or may grow into a tissue, organ, or a multicellular organism. Transformed nucleic acid molecules of the present invention can remain extrachromosomal or can integrate into one or more sites within a chromosome of the transformed (i.e., recombinant) cell in such a manner that their ability to be expressed is retained. Preferred nucleic acid molecules with which to transform a cell include asparaginase nucleic acid molecules disclosed herein. Particularly preferred nucleic acid molecules with which to transform a cell include nDiAsp₁₇₅₃, nDiAsp₁₅₁₈, nDiAsp₄₃₉, nDiAsp₃₆₉, nDiAsp₁₇₇₀, and nDiAsp₂₀₇₃.

Suitable host cells to transform include any cell that can be transformed with a nucleic acid molecule of the present invention. Host cells can be either untransformed cells or cells that are already transformed with at least one nucleic acid molecule (e.g., nucleic acid molecules encoding one or more proteins of the present invention or encoding other proteins useful in the production of multivalent vaccines). A recombinant cell of the present invention can be endogenously (i.e., naturally) capable of producing a parasitic helminth asparaginase protein of the present invention or can be capable of producing such a protein after being transformed with at least one nucleic acid molecule of the present invention. A host cell of the present invention can be any cell capable of producing at least one protein of the present invention, and can be a bacterial, fungal (including yeast), parasite (including helminth, protozoa and ectoparasite), other insect, other animal or plant cell. Preferred host cells include bacterial, mycobacterial, yeast, helminth, insect and mammalian cells. More preferred host cells include *Salmonella*, *Escherichia*, *Bacillus*, *Listeria*, *Saccharomyces*, *Spodoptera*, *Mycobacteria*, *Trichoplusia*, BHK (baby hamster kidney) cells, MDCK cells (Madin-Darby Canine Kidney cells), CRFK cells (Crandell Feline

Kidney cells), BSC-1 cells (African monkey kidney cell line used, for example, to culture poxviruses), COS (e.g., COS-7) cells, and Vero cells. Particularly preferred host cells are *Escherichia coli*, including *E. coli* K-12 derivatives; *Salmonella typhi*; *Salmonella typhimurium*, including attenuated strains such as UK-1³⁹⁸⁷ and SR-11⁴⁰⁷²; *Spodoptera frugiperda*; *Trichoplusia ni*; BHK cells; MDCK cells; CRFK cells; BSC-1 cells; COS cells; Vero cells; and non-tumorigenic mouse myoblast G8 cells (e.g., ATCC CRL 1246). Additional appropriate mammalian cell hosts include other kidney cell lines, other fibroblast cell lines (e.g., human, murine or chicken embryo fibroblast cell lines), myeloma cell lines, Chinese hamster ovary cells, mouse NIH/3T3 cells, LMTK³¹ cells and/or HeLa cells. In one embodiment, the proteins may be expressed as heterologous proteins in myeloma cell lines employing immunoglobulin promoters.

A recombinant cell of the present invention includes any cell transformed with at least one of any nucleic acid molecule of the present invention. Suitable and preferred nucleic acid molecules as well as suitable and preferred recombinant molecules with which to transform such a cell are disclosed herein. A particularly preferred recombinant cell is *E. coli*:PHis-DiAsp₁₇₇₀.

In one embodiment, a recombinant cell of the present invention can be co-transformed with a recombinant molecule including a parasitic helminth asparaginase nucleic acid molecule encoding a protein of the present invention and a nucleic acid molecule encoding another protective compound, as disclosed herein (e.g., to produce multivalent vaccines).

Recombinant DNA technologies can be used to improve expression of a transformed nucleic acid molecule by manipulating, for example, the number of copies of the nucleic acid molecule within a host cell, the efficiency with which that nucleic acid molecule is transcribed, the efficiency with which the resultant transcript is translated, and the efficiency of post-translational modifications. Recombinant techniques useful for increasing the expression of a nucleic acid molecule of the present invention include, but are not limited to, operatively linking the nucleic acid molecule to a high-copy number plasmid, integration of the nucleic acid molecule into one or more host cell chromosomes, addition of vector stability sequences to a plasmid, substitution or modification of transcription control signals (e.g., promoters, operators, enhancers), substitution or modification of translational control signals (e.g., ribosome binding sites, Shine-Dalgarno sequences, or Kozak sequences), modification of a nucleic acid molecule of the present invention to correspond to the codon usage of the host cell, deletion of sequences that destabilize transcripts, and the use of control signals that temporally separate recombinant cell growth from recombinant enzyme production during fermentation. The activity of an expressed recombinant protein of the present invention may be improved by fragmenting, modifying, or derivatizing a nucleic acid molecule encoding such a protein.

Isolated parasitic helminth asparaginase proteins of the present invention can be produced in a variety of ways, including production and recovery of natural proteins, production and recovery of recombinant proteins, and chemical synthesis of the proteins. In one embodiment, an isolated protein of the present invention is produced by culturing a cell capable of expressing the protein under conditions effective to produce the protein, and recovering the protein. A preferred cell to culture is a recombinant cell of the present invention. Effective culture conditions include, but

are not limited to, effective media, bioreactor, temperature, pH and oxygen conditions that permit protein production. An effective medium refers to any medium in which a cell is cultured to produce a parasitic helminth asparaginase protein of the present invention. Such a medium typically comprises an aqueous base having assimilable carbon, nitrogen and phosphate sources, and appropriate salts, minerals, metals and other nutrients, such as vitamins. Cells of the present invention can be cultured in conventional fermentation bioreactors, shake flasks, test tubes, microtiter dishes, and petri plates. Culturing can be carried out at a temperature, pH and oxygen content appropriate for a given recombinant cell. Such culturing conditions are within the expertise of one of ordinary skill in the art. Examples of suitable conditions are included in the Examples section.

Depending on the vector and host system used for production, a resultant protein of the present invention may either remain within the recombinant cell; be secreted into the fermentation medium; be secreted into a space between two cellular membranes, such as the periplasmic space in *E. coli*; or be retained on the outer surface of a cell or viral membrane.

The phrase "recovering the protein", as well as similar phrases, refer to collecting the whole fermentation medium containing the protein and need not imply additional steps of separation or purification. Proteins of the present invention can be purified using a variety of standard protein purification techniques, such as, but not limited to, affinity chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, reverse phase chromatography, concanavalin A chromatography, chromatofocusing and differential solubilization. Proteins of the present invention are preferably retrieved in "substantially pure" form. As used herein, "substantially pure" refers to a purity that allows for the effective use of the protein as a therapeutic composition or diagnostic. A therapeutic composition for animals, for example, should exhibit no substantial toxicity and preferably should be capable of stimulating the production of antibodies in a treated animal.

The present invention also includes isolated (i.e., removed from their natural milieu) antibodies that selectively bind to a parasitic helminth asparaginase protein of the present invention or a mimotope thereof (e.g., anti-parasitic helminth asparaginase antibodies). As used herein, the term "selectively binds to" an asparaginase protein refers to the ability of an antibody of the present invention to preferentially bind to specified proteins and mimitopes thereof of the present invention. Binding can be measured using a variety of methods standard in the art including enzyme immunoassays (e.g., ELISA), immunoblot assays, etc. See, for example, Sambrook et al., *ibid.*, and Harlow, et al., 1988, *Antibodies, a Laboratory Manual*, Cold Spring Harbor Labs Press; Harlow et al., *ibid.*, is incorporated by reference herein in its entirety. An anti-parasitic helminth asparaginase antibody preferably selectively binds to a parasitic helminth asparaginase protein in such a way as to reduce the activity of that protein.

Isolated antibodies of the present invention can include antibodies in serum, or antibodies that have been purified to varying degrees. Antibodies of the present invention can be polyclonal or monoclonal, functional equivalents such as antibody fragments and genetically-engineered antibodies, including single chain antibodies or chimeric antibodies that can bind to more than one epitope.

A preferred method to produce antibodies of the present invention includes (a) administering to an animal an effec-

tive amount of a protein, peptide or mimetope thereof of the present invention to produce the antibodies and (b) recovering the antibodies. In another method, antibodies of the present invention are produced recombinantly using techniques as heretofore disclosed to produce asparaginase proteins of the present invention. Antibodies raised against defined proteins or mimetopes can be advantageous because such antibodies are not substantially contaminated with antibodies against other substances that might otherwise cause interference in a diagnostic assay or side effects if used in a therapeutic composition.

Antibodies of the present invention have a variety of potential uses that are within the scope of the present invention. For example, such antibodies can be used (a) as therapeutic compounds to passively immunize an animal in order to protect the animal from parasitic helminths susceptible to treatment by such antibodies, (b) as reagents in assays to detect infection by such helminths or (c) as tools to screen expression libraries or to recover desired proteins of the present invention from a mixture of proteins and other contaminants. Furthermore, antibodies of the present invention can be used to target cytotoxic agents to parasitic helminths of the present invention in order to directly kill such helminths. Targeting can be accomplished by conjugating (i.e., stably joining) such antibodies to the cytotoxic agents using techniques known to those skilled in the art. Suitable cytotoxic agents are known to those skilled in the art.

One embodiment of the present invention is a therapeutic composition that, when administered to an animal in an effective manner, is capable of protecting that animal from disease caused by a parasitic helminth. A therapeutic composition of the present invention includes an excipient and at least one of the following protective compounds: an isolated native parasitic helminth asparaginase protein; an isolated non-native parasitic helminth asparaginase protein; a mimetope of a parasitic helminth asparaginase protein; an isolated parasitic helminth asparaginase nucleic acid molecule; an isolated antibody that selectively binds to a parasitic helminth asparaginase protein; or an inhibitor of asparaginase protein activity identified by its ability to inhibit parasitic helminth asparaginase activity. As used herein, a protective compound refers to a compound that, when administered to an animal in an effective manner, is able to treat, ameliorate, or prevent disease caused by a parasitic helminth. Preferred helminths to target are heretofore disclosed. Examples of proteins, nucleic acid molecules, antibodies and inhibitors of the present invention are disclosed herein.

The present invention also includes a therapeutic composition comprising at least one parasitic helminth asparaginase-based compound of the present invention in combination with at least one additional compound protective against one or more infectious agents. Examples of such compounds and infectious agents are disclosed herein.

A therapeutic composition of the present invention can be administered to any animal susceptible to such therapy, preferably to mammals, and more preferably to dogs, cats, humans, ferrets, horses, cattle, sheep and other pets, work animals, economic food animals, or zoo animals. Preferred animals to protect against heartworm disease include dogs, cats, humans and ferrets, with dogs and cats being particularly preferred.

In one embodiment, a therapeutic composition of the present invention can be administered to the vector in which the parasitic helminth develops, such as to a mosquito, in order to prevent the spread of parasitic helminth to the

definitive mammalian host. Such administration could be orally or by developing transgenic vectors capable of producing at least one therapeutic composition of the present invention. In another embodiment, a vector, such as a mosquito, can ingest therapeutic compositions present in the blood of a host that has been administered a therapeutic composition of the present invention.

A therapeutic composition of the present invention can be formulated in an excipient that the animal to be treated can tolerate. Examples of such excipients include water, saline, Ringer's solution, dextrose solution, Hank's solution, and other aqueous physiologically balanced salt solutions. Non-aqueous vehicles, such as fixed oils, sesame oil, ethyl oleate, or triglycerides may also be used. Other useful formulations include suspensions containing viscosity enhancing agents, such as sodium carboxymethylcellulose, sorbitol, or dextran. Excipients can also contain minor amounts of additives, such as substances that enhance isotonicity and chemical stability. Examples of buffers include phosphate buffer, bicarbonate buffer, and Tris buffer, while examples of preservatives include thimerosal,—or o-cresol, formalin, and benzyl alcohol. Standard formulations can either be liquid injectables or solids which can be taken up in a suitable liquid as a suspension or solution for injection. Thus, in a non-liquid formulation, the excipient can comprise dextrose, human serum albumin, preservatives, etc., to which sterile water or saline can be added prior to administration.

In one embodiment of the present invention, a therapeutic composition can include an adjuvant. Adjuvants are agents that are capable of enhancing the immune response of an animal to a specific antigen. Suitable adjuvants include, but are not limited to, cytokines, chemokines, and compounds that induce the production of cytokines and chemokines (e.g., granulocyte macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF), colony stimulating factor (CSF), erythropoietin (EPO), interleukin 2 (IL-2), interleukin-3 (IL-3), interleukin 4 (IL-4), interleukin 5 (IL-5), interleukin 6 (IL-6), interleukin 7 (IL-7), interleukin 8 (IL-8), interleukin 10 (IL-10), interleukin 12 (IL-12), interferon gamma, interferon gamma inducing factor I (IGIF), transforming growth factor beta, RANTES (regulated upon activation, normal T-cell expressed and presumably secreted), macrophage inflammatory proteins (e.g., MIP-1 alpha and MIP-1 beta), and Leishmania elongation initiating factor (LEIF)); bacterial components (e.g., endotoxins, in particular superantigens, exotoxins and cell wall components); aluminum-based salts; calcium-based salts; silica; polynucleotides; toxoids; serum proteins, viral coat proteins; block copolymer adjuvants (e.g., Hunter's Titermax™ adjuvant (Vaxcel™, Inc. Norcross, Ga.), Ribi adjuvants (Ribi ImmunoChem Research, Inc., Hamilton, Mont.); and saponins and their derivatives (e.g., Quil A (Superfos Biosector A/S, Denmark). Protein adjuvants of the present invention can be delivered in the form of the protein themselves or of nucleic acid molecules encoding such proteins using the methods described herein.

In one embodiment of the present invention, a therapeutic composition can include a carrier. Carriers include compounds that increase the half-life of a therapeutic composition in the treated animal. Suitable carriers include, but are not limited to, polymeric controlled release vehicles, biodegradable implants, liposomes, bacteria, viruses, other cells, oils, esters, and glycols.

One embodiment of the present invention is a controlled release formulation that is capable of slowly releasing a composition of the present invention into an animal. As used

herein, a controlled release formulation comprises a composition of the present invention in a controlled release vehicle. Suitable controlled release vehicles include, but are not limited to, biocompatible polymers, other polymeric matrices, capsules, microcapsules, microparticles, bolus preparations, osmotic pumps, diffusion devices, liposomes, lipospheres, and transdermal delivery systems. Other controlled release formulations of the present invention include liquids that, upon administration to an animal, form a solid or a gel in situ. Preferred controlled release formulations are biodegradable (i.e., bioerodible).

A preferred controlled release formulation of the present invention is capable of releasing a composition of the present invention into the blood of the treated animal at a constant rate sufficient to attain therapeutic dose levels of the composition to protect an animal from disease caused by parasitic helminths. The therapeutic composition is preferably released over a period of time ranging from about 1 to about 12 months. A controlled release formulation of the present invention is capable of effecting a treatment preferably for at least about 1 month, more preferably for at least about 3 months, even more preferably for at least about 6 months, even more preferably for at least about 9 months, and even more preferably for at least about 12 months.

In order to protect an animal from disease caused by a parasitic helminth, a therapeutic composition of the present invention is administered to the animal in an effective manner such that the composition is capable of protecting that animal from a disease caused by a parasitic helminth. For example, an isolated protein or mimotope thereof is administered in an amount and manner that elicits (i.e., stimulates) an immune response that is sufficient to protect the animal from the disease. Similarly, an antibody of the present invention, when administered to an animal in an effective manner, is administered in an amount so as to be present in the animal at a titer that is sufficient to protect the animal from the disease, at least temporarily. An oligonucleotide nucleic acid molecule of the present invention can also be administered in an effective manner, thereby reducing expression of native parasitic helminth asparaginase proteins in order to interfere with development of the parasitic helminths targeted in accordance with the present invention.

Therapeutic compositions of the present invention can be administered to animals prior to infection in order to prevent infection (i.e., as a preventative vaccine) or can be administered to animals after infection in order to treat disease caused by the parasitic helminth (i.e., as a curative agent or a therapeutic vaccine).

Acceptable protocols to administer therapeutic compositions in an effective manner include individual dose size, number of doses, frequency of dose administration, and mode of administration. Determination of such protocols can be accomplished by those skilled in the art. A suitable single dose is a dose that is capable of protecting an animal from disease when administered one or more times over a suitable time period. For example, a preferred single dose of a protein, mimotope, or antibody therapeutic composition is from about 1 microgram (μg) to about 10 milligrams (mg) of the therapeutic composition per kilogram body weight of the animal. Booster vaccinations can be administered from about 2 weeks to several years after the original administration. Booster administrations preferably are administered when the immune response of the animal becomes insufficient to protect the animal from disease. A preferred administration schedule is one in which from about 10 μg to about 1 mg of the therapeutic composition per kg body weight of the animal is administered from about one to about two

times over a time period of from about 2 weeks to about 12 months. Modes of administration can include, but are not limited to, subcutaneous, intradermal, intravenous, intranasal, oral, transdermal, and intramuscular routes.

According to one embodiment, a nucleic acid molecule of the present invention can be administered to an animal in a fashion to enable expression of that nucleic acid molecule into a protective protein or protective RNA (e.g., an anti-sense RNA, a ribozyme, a triple helix form, or an RNA drug) in the animal. Nucleic acid molecules can be delivered to an animal by a variety of methods including, but not limited to, (a) administering a genetic vaccine (e.g., a naked DNA or RNA molecule, such as is taught, for example, in Wolff et al., 1990, *Science* 247, 1465-1468) or (b) administering a nucleic acid molecule packaged as a recombinant virus vaccine or as a recombinant cell vaccine (i.e., the nucleic acid molecule is delivered by a viral or cellular vehicle).

A genetic (i.e., naked nucleic acid) vaccine of the present invention includes a nucleic acid molecule of the present invention and preferably includes a recombinant molecule of the present invention that preferably is replication, or otherwise amplification, competent. A genetic vaccine of the present invention can comprise one or more nucleic acid molecules of the present invention in the form of, for example, a dicistronic recombinant molecule. A preferred genetic vaccine includes at least a portion of a viral genome (i.e., a viral vector). Preferred viral vectors include those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, picornaviruses, and retroviruses, with those based on alphaviruses (such as Sindbis or Semliki forest virus), species-specific herpesviruses and poxviruses being particularly preferred. Any suitable transcription control sequence can be used, including those disclosed as suitable for protein production. Particularly preferred transcription control sequences include cytomegalovirus immediate early (preferably in conjunction with Intron-A), Rous sarcoma virus long terminal repeat, and tissue-specific transcription control sequences, as well as transcription control sequences endogenous to viral vectors if viral vectors are used. The incorporation of "strong" poly(A) sequences is also preferred.

A genetic vaccine of the present invention can be administered in a variety of ways, with intramuscular, subcutaneous, intradermal, transdermal, intranasal and oral routes of administration being preferred. A preferred single dose of a genetic vaccine ranges from about 1 nanogram (ng) to about 500 μg , depending on the route of administration or method of delivery, as can be determined by those skilled in the art. Suitable delivery methods include, for example, by injection, as drops, aerosolized, or topically. Genetic vaccines of the present invention can be contained in an aqueous excipient (e.g., phosphate buffered saline) alone or in a carrier (e.g., lipid-based vehicles).

A recombinant virus vaccine of the present invention includes a recombinant molecule of the present invention that is packaged in a viral coat and that can be expressed in an animal after administration. Preferably, the recombinant molecule is packaging- or replication-deficient or encodes an attenuated virus. A number of recombinant viruses can be used, including, but not limited to, those based on alphaviruses, poxviruses, adenoviruses, herpesviruses, picornaviruses, and retroviruses. Preferred recombinant virus vaccines are those based on alphaviruses (such as Sindbis virus), raccoon poxviruses, picornaviruses, and species-specific herpesviruses. Methods to produce and use a recombinant alphavirus vaccine are disclosed in PCT Publication No. WO 94/17813, by Xiong et al., published Aug. 18, 1994, which is incorporated by reference herein in its entirety.

When administered to an animal, a recombinant virus vaccine of the present invention infects cells within the immunized animal and directs the production of a protective protein or RNA nucleic acid molecule that is capable of protecting the animal from disease caused by a parasitic helminth as disclosed herein. For example, a recombinant virus vaccine comprising a parasitic helminth asparaginase nucleic acid molecule of the present invention is administered according to a protocol that results in the animal producing a sufficient immune response to protect itself from heartworm disease. A preferred single dose of a recombinant virus vaccine of the present invention is from about 1×10^4 to about 1×10^8 virus plaque forming units (pfu) per kilogram body weight of the animal. Administration protocols are similar to those described herein for protein-based vaccines, with subcutaneous, intramuscular, intranasal and oral administration routes being preferred.

A recombinant cell vaccine of the present invention includes a recombinant cell of the present invention that expresses at least one protein of the present invention. Preferred recombinant cells for this embodiment include *Salmonella*, *E. coli*, *Listeria*, *Mycobacterium*, *S. frugiperda*, yeast (including *Saccharomyces cerevisiae* and *Pichia pastoris*), BHK, BSC-1, myoblast G8, COS (e.g., COS-7), Vero, MDCK or CRFK recombinant cells. A recombinant cell vaccine of the present invention can be administered in a variety of ways but has the advantage that it can be administered orally, preferably at doses ranging from about 10^8 to about 10^{12} cells per kilogram body weight. Administration protocols are similar to those described herein for protein-based vaccines. A recombinant cell vaccine can comprise whole cells, cells stripped of cell walls or cell lysates.

The efficacy of a therapeutic composition of the present invention to protect an animal from disease caused by a parasitic helminth can be tested in a variety of ways including, but not limited to, detection of protective antibodies (using, for example, proteins or mimetopes of the present invention), detection of cellular immunity within the treated animal, or challenge of the treated animal with the parasitic helminth to determine whether the treated animal is resistant to disease. Challenge studies can include implantation of chambers including parasitic helminth larvae into the treated animal and/or direct administration of larvae to the treated animal. In one embodiment, therapeutic compositions can be tested in animal models such as mice. Such techniques are known to those skilled in the art.

One preferred embodiment of the present invention is the use of parasitic helminth asparaginase proteins, nucleic acid molecules, antibodies or inhibitory compounds of the present invention to protect an animal from heartworm disease. It is particularly preferred to prevent L3 that are delivered to the animal by the mosquito intermediate host from maturing into adult worms. Accordingly, a preferred therapeutic composition is one that is able to inhibit at least one step in the portion of the parasite's development cycle that includes L3, third molt, L4, fourth molt, and immature adult prior to entering the circulatory system. In dogs, this portion of the developmental cycle is about 70 days in length. A particularly preferred therapeutic composition includes a parasitic helminth asparaginase-based therapeutic composition of the present invention, particularly in light of the evidence herein reported that asparaginase is expressed in both larval and adult stages of the parasite. Such a composition can include a parasitic helminth asparaginase nucleic acid molecule, a parasitic helminth asparaginase protein or a mimetope thereof, anti-parasitic helminth

asparaginase antibodies, or inhibitors of parasitic helminth asparaginase activity. Such therapeutic compositions are administered to an animal in a manner effective to protect the animals from heartworm disease. Additional protection may be obtained by administering additional protective compounds, including other parasitic helminth proteins, nucleic acid molecules, antibodies and inhibitory compounds, as disclosed herein.

One therapeutic composition of the present invention includes an inhibitor of parasitic helminth asparaginase activity, i.e., a compound capable of substantially interfering with the function of a parasitic helminth asparaginase protein, also referred to herein as an asparaginase inhibitor. In one embodiment, such an inhibitor comprises a compound that interacts directly with an asparaginase protein active site (usually by binding to or modifying the active site), thereby inhibiting asparaginase activity. According to this embodiment, an asparaginase inhibitor can also interact with other regions of an asparaginase protein to inhibit asparaginase activity, for example, by allosteric interaction. Preferably, an asparaginase inhibitor of the present invention is identified by its ability to bind to, or otherwise interact with, a parasitic helminth asparaginase protein, thereby inhibiting asparaginase activity of that protein. Such an asparaginase inhibitor is a suitable for inclusion in a therapeutic composition of the present invention as long as the compound is not harmful to the host animal being treated.

An asparaginase inhibitor can be identified using a parasitic helminth asparaginase protein of the present invention. As such, one embodiment of the present invention is a method to identify a compound capable of inhibiting asparaginase activity of a parasitic helminth susceptible to inhibition by an inhibitor of parasitic helminth asparaginase activity. Such a method includes the steps of (a) contacting (e.g., combining, mixing) an isolated parasitic helminth asparaginase protein, preferably a *D. immitis* asparaginase protein, with a putative inhibitory compound under conditions in which, in the absence of the compound, the protein has asparaginase activity, and (b) determining if the putative inhibitory compound inhibits the asparaginase activity. Putative inhibitory compounds to screen include small organic molecules, antibodies (including mimetopes thereof) and substrate analogs. Methods to determine asparaginase activity are known to those skilled in the art; see, for example, Rhee, et al., *ibid.*, Lim, et al., *ibid.*, Sauri, et al., *ibid.*, and Kim, et al., *ibid.*

The present invention also includes a test kit to identify a compound capable of inhibiting asparaginase activity of a parasitic helminth. Such a test kit includes an isolated parasitic helminth asparaginase protein, preferably a *D. immitis* asparaginase protein, having asparaginase activity, and a means for determining the extent of inhibition of asparaginase activity in the presence of (i.e., effected by) a putative inhibitory compound. Such compounds are also screened to identify those that are substantially not toxic in host animals, e.g., compounds that do not inhibit the activity of mammalian asparaginase.

Asparaginase inhibitors isolated by such a method or test kit can be used to inhibit any parasitic helminth asparaginase protein that is susceptible to such an inhibitor. A particularly preferred asparaginase inhibitor of the present invention is capable of protecting an animal from heartworm disease. A therapeutic composition comprising a compound that inhibits asparaginase activity can be administered to an animal in an effective manner to protect that animal from disease caused by the parasite expressing the targeted asparaginase enzyme, and preferably to protect that animal from heart-

worm disease. Effective amounts and dosing regimens can be determined using techniques known to those skilled in the art.

It is also within the scope of the present invention to use isolated proteins, mimetopes, nucleic acid molecules and antibodies of the present invention as diagnostic reagents to detect infection by parasitic helminths. Such diagnostic reagents can be supplemented with additional compounds that can detect specific phases of the parasite's life cycle. Methods to use such diagnostic reagents to diagnose parasitic helminth infection are well known to those skilled in the art. Suitable and preferred parasitic helminths to detect are those to which therapeutic compositions of the present invention are targeted. Particularly preferred parasitic helminths to detect using diagnostic reagents of the present invention are *Dirofilaria*.

The following examples are provided for the purposes of illustration and are not intended to limit the scope of the present invention.

EXAMPLES

It is to be noted that these Examples include a number of molecular biology, microbiology, immunology and biochemistry techniques familiar to those skilled in the art. Disclosure of such techniques can be found, for example, in Sambrook et al., *ibid.*, Ausubel, et al., 1993, *Current Protocols in Molecular Biology*, Greene/Wiley Interscience, New York, N.Y., and related references. Ausubel, et al., *ibid.* is incorporated by reference herein in its entirety. DNA and protein sequence analyses were carried out using the PC/GENE™ sequence analysis program (available from Intelligenetics, Inc., Mountainview, Calif.) and the Wisconsin Package™ Version 9.0 (available from the Genetics Computer Group (GCG), Madison, Wis.). It should also be noted that, because nucleic acid sequencing technology, and in particular the sequencing of PCR products, is not entirely error-free, the nucleic acid and deduced protein sequences presented herein represent apparent nucleic acid sequences of the nucleic acid molecules encoding parasitic helminth asparaginase proteins of the present invention.

Example 1

This Example describes the collection of *D. immitis* L3 cuticles, preparation of cuticular antigen, and generation of polyclonal antibodies to L3 cuticle.

L₃ cuticle collection:

Infective stage larvae (L3) collected from mosquitoes were washed three times in NI medium (equal volumes of NCTC-135 and IMDM, available from Sigma Chemical Co., St. Louis, Mo.), then resuspended in NI medium supplemented with 20% SeruMax™ (available from Sigma Chemical Co.). The washed larvae were cultured at a density of 250–440 larvae per ml, with 10 ml per 25 cm² flask, at 37° C. in an atmosphere of 5% CO₂ in air and 95% relative humidity. After culturing in SeruMax-supplemented medium for 48 hr to induce molting, the larvae were washed five times in serum-free medium and then cultured for an additional 4 days. On day 6, the flasks were held at a 45° angle for 20 min to settle the molted L4 larvae. The medium containing the cuticles (which are relatively low density, and therefore float in the culture medium) was then drawn out of the flasks into a 15 ml centrifuge tube. The medium was then spun at 3500 RPM for 15 min to pellet all cuticles. The flasks containing the L4 larvae were resuspended in 5 ml of PBS containing 0.1% Triton X-100, and the above process was repeated two more times to collect cuticles that had settled

with the L4 larvae. All cuticle preparations were then pooled and stored at –70° C. until use.

Anti-cuticle antisera:

33,000 L3 cuticles were homogenized in 1 ml of extraction buffer (20 mM Tris/HCl pH 8.5, containing 2 mM 1,4-dithiothreitol, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM N-tosyl-L-lysine chloromethane and 0.1 mM N-tosyl-L-phenylalanine chloromethane and 0.1% Triton X-100) for 20 min on ice. The crude extract thus obtained was sonicated for three, one min periods, with a five min rest following each minute of sonication, using a pre-chilled small probe of a W-380 Ultrasonic Processor (available from Heat Systems-Ultrasonics, Farmingdale, N.Y.). The cuticle extract was then stored at –70° C. until use.

Twelve mice were immunized subcutaneously, first with approximately 15 μl of larval cuticle extract (approximately 500 larval cuticles) with complete Freund's Adjuvant, and then with three subsequent immunizations of the same dose of extract mixed in incomplete Freund's adjuvant. Immunizations were performed on days 14, 28, 61 and 104. Mice were bled on days 0, 21, 35, 50, 75, 83, 91, 98, 105, 114, 121, 134, and 140, and the cellular blood components were separated from the sera by centrifugation. The sera, referred to herein as anti-cuticle antisera, were stored at –70° C. until use.

An immunoglobulin G- (IgG-) enriched fraction from the anti-cuticle antisera (collected at day 114 post first immunization) was prepared by 50% ammonium sulfate precipitation. This IgG-enriched preparation is referred to herein as IgG-enriched anti-cuticle antisera. Ammonium ions were removed by extensive dialysis in 0.1M PBS, pH 7.2. The IgG content was determined by measuring absorbance at OD₂₈₀ versus a blank PBS control. IgG from pre-immune mice sera (day 0) was prepared in a similar manner.

Example 2

This Example demonstrates the immunoreactivity (as determined by ELISA) of anti-cuticle antisera.

Total IgG, IgM and IgG subclass antibodies to L3 soluble antigens were measured in sera from mice immunized with L3 cuticles (anti-cuticle antisera, as described above). Crude extracts of *D. immitis* infective stage larvae (L3) were prepared as follows. Larval heartworm parasites were homogenized in buffer B (20 mM Tris/HCl pH 8.5, containing 2 mM 1,4-dithiothreitol, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 0.1 mM N-tosyl-L-lysine chloromethane and 0.1 mM N-tosyl-L-phenylalanine chloromethane; all available from Sigma) for 20 min on ice. The crude extracts thus obtained were sonicated continuously for three 1-min periods, with 5-min intervals between each sonication, using a pre-chilled small probe of the W-380 Ultrasonic Processor (available from Heat Systems-Ultrasonics, Farmingdale, N.Y.). The third sonication was done in the presence of 0.1% Triton X-100. The suspensions were centrifuged at 15,000×g for 20 min. The supernatants thus obtained (referred to herein as the parasite extracts, or crude parasite extracts) were diluted to 1.0 μg protein/ml in 0.06M carbonate buffer, pH 9.6, and then incubated overnight at 4° C. in Immulon®2 microtiter plates (available from Dynatech Laboratories, Alexandria, Va.), 100 μl/well. The plates were blocked with 0.01M PBS (pH 7.4) containing 0.05% Tween 20 and 5% fetal calf serum (PBS/T/FCS) for 1 hr at 37° C. Serum samples from mice immunized with L3 cuticular antigens (anti-cuticle antisera, prepared as

described in Example 1) were diluted 1:25 in PBS/T/FCS and were added to the first row of the ELISA plates. Two-fold dilutions were carried out throughout the remaining rows. After 1 hr incubation at 37° C., the plates were washed with PBS/T, and antibody binding was detected with peroxidase-conjugated anti-mouse total IgG, IgG₁, IgG_{2a}, IgG_{2b}, IgG₃ and IgM antibodies, respectively (available from Kirkegaard and Perry Ltd., Gaithersburg, Md.). After 1 hr incubation, the plates were washed and o-phenyldiamine/H₂O₂ substrate was added (available from Amresco®, Solon, Ohio). The enzyme reaction was stopped after 5 min at room temperature with 4M H₂SO₄. The optical density (OD) was read relative to a PBS blank at 490 nm with an ELISA reader (for example, a SpectraMax™ 250, available from Molecular Devices, Sunnyvale, Calif.). A strong total IgG response to L3 soluble antigens in sera from mice immunized with L3 cuticles was observed at day 21 post first immunization. The main IgG subclass that was elevated in these mice was IgG₁ isotype. In addition, there was a detectable IgG_{2b} isotype response in these mice to cuticular antigens during the latter part of the immunization schedule (by day 75). Elevated levels of total IgM antibodies were also detected in sera from these mice. These results are presented in Table 1, which shows the optical density of the wells incubated with a 1:400 dilution of anti-cuticle antisera.

TABLE 1

DAYS POST-INITIAL IMMUNIZATION: ISOTYPE:													
	0	21	35	50	75	83	91	98	105	114	121	134	140
total IgG	0.000	0.689	2.146	2.497	3.639	3.608	3.812	3.821	3.650	4.027	3.858	3.792	3.897
IgG ₁	0.002	0.340	1.437	1.756	2.883	3.089	3.313	2.980	2.682	3.002	2.996	2.706	3.032
IgG _{2a}	0.007	0.030	0.107	0.119	0.229	0.211	0.279	0.218	0.190	0.221	0.200	0.193	0.213
IgG _{2b}	0.007	0.074	0.248	0.370	0.771	0.723	0.729	0.600	0.608	0.702	0.742	0.607	0.654
IgG ₃	0.000	0.004	0.002	0.001	0.002	0.000	0.008	0.006	0.001	0.002	0.003	0.000	0.003
IgM	0.025	1.615	2.700	2.009	1.762	1.872	1.847	2.267	1.968	3.010	2.707	2.486	2.849

Example 3

This Example describes quantitation of antibodies to *D. immitis* larval surfaces in sera from mice immunized with L3 cuticles.

Antibodies to larval surfaces in anti-cuticle antisera were quantitated by an indirect fluorescent antibody assay (IFA). Infection of mosquitoes and collection of 0-hr L3 (mosquito-derived infective stage larvae) and 48-hr L3 (48-hr after in vitro culture) were carried out as previously described (Frank, G. R. and Grieve, R. B., 1991, *J Parasitol.* 77, 950–956). Each larval stage was processed separately. Larvae were fixed for 24 hr at 4° C. in 4% formalin in PBS. After centrifugation at 7,000×g for 1 min, 50 larvae per tube were washed once in either PBS (for 0-hr L3 and 48-h L3) or with 0.1% Triton X-100 in PBS for L4 larvae collected 6 days post initial culture. Fifty μl of a 1:4 dilution of IgG-enriched anti-cuticle antisera in PBS was added to the resulting larval pellet and incubated at 4° C. overnight. After three washes as described above, 50 μl of 1:20 dilution of the F(ab)₂ fraction of a fluorescein-conjugated goat anti-mouse IgG (available from Kirkegaard and Perry, Ltd.) was added to the pellet. This preparation was incubated overnight at 4° C., after which the larvae were again washed three times. The resulting larval pellet was resuspended in 50 μl of a 25% glycerol mixture in PBS containing 0.1% p-phenylenediamine. A wet mount of this suspension was placed under a cover slip and observed at 400× using a

Model BH-2 Olympus microscope equipped with an exciter IF-490 filter, DM-500 (0–515) dichroic mirror, and a mercury 100 W lamp (available from Olympus Optical Company, Ltd., Tokyo, Japan). A Nikon PI (UFX-11) photometer system equipped with an IF-530 filter and a 2.0 mm diaphragm, was used to quantitate fluorescence (available from Nikon Corporation, Tokyo, Japan). To measure antibody levels to larval surface antigens, three second readings were taken on three representative areas of fluorescence on each of five worms for each serum sample (IgG-enriched anti-cuticle antisera prepared from day 0 and day 114 post immunization), and the highest number within each three second determination was recorded. Data for each serum sample are presented in Table 2 as the average of the fluorescence values of three representative areas on each of five L3 larvae. IgG-enriched anti-cuticle antisera, prepared as described in Example 1, strongly immunoreacted with surface antigens both in 0-hr and 48-hr L3. Pre-immune mouse serum failed to react with larval surface antigens.

TABLE 2

Reactivity* of IgG-enriched anti-cuticle antisera to *D. immitis* larval surface antigens

Larval stage	Relative fluorescence units [†]	
	Mouse pre-bleed IgG	Mouse anti-cuticle IgG
0-hr L3	1.1 ± 0.1	33.3 ± 3.6
48-hr L3	1.7 ± 0.2	13.1 ± 13.2

*Reactivity of IgG-enriched anti-cuticle antisera to *D. immitis* larval surface antigens was measured by indirect fluorescent antibody assay as described in the text.

[†]Data represent Mean ± SD of at least 3 readings per larva (n = 5)

Example 4

This Example describes the isolation and sequencing of *D. immitis* asparaginase (DiASNase) nucleic acid molecules of present invention.

A DiASNase nucleic acid molecule of 1753 nucleotides, herein referred to as nDiASNase₁₇₅₃, was cloned from a *D. immitis* larval cDNA library by immunoscreening. Specifically, a *D. immitis* 48-hr L3 cDNA expression library was constructed in Uni-ZAP™ XR vector (available from Stratagene Cloning Systems, La Jolla, Calif.), using a ZAP-cDNA Synthesis Kit (available from Stratagene) and 48-hr L4 mRNAs. The library was immunoscreened using the IgG-enriched anti-cuticle antisera described in Example 1, and standard immunoscreening procedures as described, for example, in Sambrook et al., *ibid.* Briefly, phage were plated onto a lawn of *E. coli* XL1-Blue MRF' (available from

Stratagene) at a density of 25×10^3 phage per petri dish (150 mm^2) and grown at 37° C . for 4 hr. When plaques were visible, isopropyl- β -D thiogalactoside (IPTG)-impregnated nitrocellulose filters were placed on the plates for 3 hr at 37° C . The filters were then removed and washed in 0.01% M phosphate-buffered saline, pH 7.4 with 0.05% Tween 20 (PBS/T), and then blocked in PBS/T containing 5% nonfat dry milk for one hr at room temperature. The filters were then incubated for 3 hr in mouse IgG-enriched anti-cuticle antisera, diluted 1:200 in PBS/T, that had been previously absorbed with *E. coli* antigens. Antibody reactivity with recombinant proteins was revealed by incubation of the filters with alkaline phosphatase-conjugated goat anti-mouse IgG antibodies (available from Kirkegaard and Perry) for 1 hr, followed by development with 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (BCIP/NBT, available from Sigma). Clones that were reactive with the sera were selected and purified by repeated cycles of immune selection.

The nucleic acid molecule nDiASNase₁₇₅₃ included in the plaque-purified cloned DNA was converted into a double stranded recombinant molecule, herein denoted as p β gal-nDiASNase₁₇₅₃, using ExAssist™ helper phage and SOLR™ *E. coli* (available from Stratagene) according to the manufacturer's protocol for in vivo excision of DNA. Double stranded plasmid DNA was prepared using an alkaline lysis protocol, such as that described in Sambrook et al., *ibid.* Due to an internal EcoRI restriction site in nDiASNase₁₇₅₃, digestion of the plasmid DNA with EcoRI and XhoI restriction endonucleases resulted in the release of two DiASNase nucleic acid molecules of 463bp and 1290bp, namely nDiASNase₄₆₃ and nDiASNase₁₂₉₀. These two nucleic acid molecules together make a DiASNase nucleic acid molecule of 1753 nucleotides in size, herein referred to as nDiASNase₁₇₅₃.

The plasmid containing nDiASNase₁₇₅₃ was sequenced by the Sanger dideoxy chain termination method, using the PRISM™ Ready Dye Terminator Cycle Sequencing Kit with AmpliTaq® DNA Polymerase, FS (available from the Perkin-Elmer Corporation, Norwalk, Conn.). PCR extensions were done in the GeneAmp™ PCR System 9600 (available from Perkin-Elmer). Excess dye terminators were removed from extension products using the Centriflex™ Gel Filtration Cartridge (available from Advanced Genetics Technologies Corporation (ABI), Gaithersburg, Md.) following the manufacturer's protocol. Samples were resuspended according to ABI protocols and then run on a Perkin-Elmer ABI PRISM™ 377 Automated DNA Sequencer. The following nucleotide primers were used to sequence nDiASNase₁₇₅₃: Three pBluescript™ vector sense primers consisting of a) a T₃X primer (denoted herein as SEQ ID NO:14) having the nucleic acid sequence, 5' AATTAACCCTCACTAAAGGG 3'; b) a M13 reverse primer (denoted herein as SEQ ID NO:15) having the nucleotide sequence, 5' GGAAACAGCTATGACCATG 3'; and c) an SK primer (denoted herein as SEQ ID NO:16) having the nucleotide sequence 5' CGCTCTAGAACTAGTGGATC 3'. In addition, two pBluescript™ vector antisense primers were used, consisting of a T₇X primer (denoted herein as SEQ ID NO:17) having the nucleotide sequence 5' GTAATACGACTCACTATAGGGC 3' and a M13 forward primer (denoted herein as SEQ ID NO:18) having the nucleotide sequence 5' GTAAAACGACGGCCAGT 3'. In addition, two nDiASNase₁₇₅₃-specific primers derived from the initial partial sequencing of nDiASNase₁₇₅₃ were used. These included a sense primer (denoted herein as SEQ ID NO:19) having the nucleotide sequence 5' CAATATTTCGT-

TCACCATCAATGGC 3', and an antisense primer (denoted herein as SEQ ID NO:20) having the nucleotide sequence 5' CGGCTCCGGCAGCAAGCCAAGAATTC 3'. These two nDiASNase₁₇₅₃-specific primers correspond to the following regions of the coding strand of nDiASNase₁₇₅₃ (herein represented by SEQ ID NO:25 (coding strand)): SEQ ID NO:19 corresponds to a region of SEQ ID NO:25 that spans from nucleotide 724 to nucleotide 748, and SEQ ID NO:20 corresponds to a region of SEQ ID NO:25 spanning from nucleotide 1610 to nucleotide 1630. The resulting nucleic acid sequences of the two complementary DNA strands of nDiASNase₁₇₅₃ are referred to herein as SEQ ID NO:1 (the coding strand) and SEQ ID NO:3 (the reverse complement of the coding strand).

Translation of SEQ ID NO:1 yields a protein of 506 amino acids, herein denoted PDiASNase₅₀₆, the amino acid sequence of which is represented by SEQ ID NO:2. The nucleic acid molecule encoding PDiASNase₅₀₆ is referred to herein as nDiASNase₁₅₁₈, the nucleic acid sequence of which is represented by SEQ ID NO:4 (the coding strand) and the SEQ ID NO:5 (the complementary strand), assuming that the first codon spans from nucleotide 1 through nucleotide 3, and a putative stop codon spans from nucleotide 1519 to nucleotide 1521 (of SEQ ID NO:1). The 3' end of the non-coding region of SEQ ID NO:1 has a polyadenylation signal, AATAAAA, spanning from nucleotide 1535 to nucleotide 1540, followed by a 20 nucleotide poly-A tail.

The amino acid sequence of PDiASNase₅₀₆ (i.e., SEQ ID NO:2) was analyzed using the PC/GENE (available from Intelligenetics, Inc., Mountain view, Calif.) sequence analysis program. The protein represented by this amino acid sequence has a predicted molecular mass of 56.4 kD and an estimated pI of 6.44. Analysis of PDiASNase₅₀₆ using the method of Hopp and Woods (Hopp and Woods, *Proc. Natl. Acad. Sci. (USA)*, 78, 3824–3828) predicts that this protein is hydrophilic.

A homology search of a non-redundant protein database was performed on SEQ ID NO:2 using the BLASTp sequence analysis program available through the BLAST™ network through the National Center for Biotechnology Information (NCBI) (National Library of Medicine, National Institute of Health, Baltimore, Md.). This database includes SwissProt+PIR+SPupdate+GenPept+GPUupdate+PDB databases. The highest scoring match of the homology search at the amino acid level was to the translation product of a *Caenorhabditis elegans* gene (C27A7.5A), GenBank™ accession number E293495. SEQ ID NO:2 was optimally aligned with the sequence represented by GenBank™ accession number E293495 using the "ALIGN" program available in the PC/GENE™ Package. The alignment revealed that a region spanning from amino acid 1 through amino acid 499 of SEQ ID NO:2 had about 54% identity to a region that spans from amino acid number 1 through amino acid 686 of the translation product of the *C. elegans* cosmid clone.

A BLASTn search of a non-redundant nucleotide database was performed using SEQ ID NO:1. At the nucleotide level, the coding region represented in SEQ ID NO:1 showed some homology to the *C. elegans* cosmid clone (C27A7.5A) nucleotide sequence, GenBank™ Accession No. Z81041. Optimal alignment using the "ALIGN" program available in the PC/GENE™ Package revealed that a region of SEQ ID NO:1, that spans from nucleotide 1 through nucleotide 1753, had about 65% identity with the nucleotide sequence of Z81041.

Example 5

This Example describes the PCR amplification and subsequent isolation of DiASNase nucleic acid molecules from

D. immitis larval and adult female first strand cDNA using a primer derived from the sequence of the nematode 22 nucleotide splice leader.

Most, but not all nematode messenger RNAs have the nematode splice leader sequence (SL1) at their 5' ends, and the presence of the 5' SL1 sequence is indicative of an apparent full length cDNA. See, for example Blaxter and Liu, 1996, *Int. J. Parasitol.* 26, 1025–1033, which is incorporated herein by reference. DiASNase nucleic acid molecules were PCR amplified from larval and adult female first strand cDNA using a sense primer representing the nematode splice leader sequence (SL1) having the nucleotide sequence, 5' GGTTTAATTACCCAAGTTTGAG 3' (denoted here in as SEQ ID NO:21). In addition, an anti-sense primer, referred to herein as ASP5'INT (represented by SEQ ID NO:22) and having the nucleotide sequence 5'GCCGTATATGCCAGTGTATCAGTACCATG 3', was used in the amplification reaction. ASP5'INT corresponds to a region of SEQ ID NO:25 that spans from nucleotide 411 through nucleotide 439. PCR amplification of larval and adult female cDNAs yielded identical 439-bp products from both larval and adult first strand cDNAs. The product amplified from adult female cDNA is referred to herein as nDiASNase₄₃₉.

Nucleic acid molecule nDiASNase₄₃₉ was gel purified, cloned into the pCR@2.1 cloning vector (available from Invitrogen, Carlsbad, Calif.) and sequenced as previously described. Sequence analysis demonstrated the presence of the SL1 sequence at the 5' end of nDiASNase₄₃₉. The coding and complementary strands of nDiASNase₄₃₉ are herein denoted as SEQ ID NO:6 and SEQ ID NO:8, respectively. Assuming an initiation codon that spans from nucleotide 69 to nucleotide 71, translation of SEQ ID NO:6 yields a protein of 123 amino acids, herein referred to as PDiASNase₁₂₃, the amino acid sequence of which is represented by SEQ ID NO:7. The coding region of PDiASNase₁₂₃ is referred to herein as nDiASNase₃₆₉, the nucleic acid sequence of which is represented in SEQ ID NO:9 (the coding strand) and SEQ ID NO:10 (the complementary strand). Analysis of the amino acid sequence of *D. immitis* PDiASNase₁₂₃ (i.e., SEQ ID NO:7) predicts that PDiASNase₁₂₃ has an estimated molecular weight of about 14.3 kD and an estimated pI of about 4.16. The fact that nucleic acid molecule nDiASNase₄₃₉ could be amplified from the larval cDNA with the SL1 primer demonstrates that the larval messenger RNA from which nDiASNase₁₇₅₃ was amplified had the 5' SL1 sequence.

A homology search of a non-redundant protein database was performed on SEQ ID NO:6 using the BLAST™ network through the National Center for Biotechnology Information (NCBI) (National Library of Medicine, National Institute of Health, Baltimore, Md.). This database includes SwissProt+PIR+SPupdate+GenPept+GPUupdate+PDB databases. The highest scoring match of the homology search at the amino acid level was to the translation product of the nucleic acid sequence of a *C. elegans* cosmid, GenBank™ accession number E293495. This homology spans from amino acid 1 through amino acid 123 of SEQ ID NO:7. Using this analysis program, the coding region represented in SEQ ID NO:6 from nucleotide 1 to nucleotide 439 was approximately 60% homologous to the sequence of a *C. elegans* cosmid, GenBank™ accession number Z81041.

A composite nucleic acid molecule representing an apparent full-length *D. immitis* asparaginase cDNA molecule was assembled by joining the overlapping nucleic acid sequences of nucleic acid molecules nDiASNase₁₇₅₃ and nDiASNase₄₃₉. This composite nucleic acid molecule is denoted

herein as nDiASNase₂₀₇₃, and has a nucleic acid sequence as represented by SEQ ID NO:25 (the coding strand) and SEQ ID NO:26 (the complementary strand). nDiASNase₂₀₇₃ encodes a protein having an amino acid sequence herein represented by SEQ ID NO:12. This protein 3 predicted N-glycosylation sites at amino acid positions 185, 192, and 297 of SEQ ID NO:12. In addition, nDiASNase₂₀₇₃ encodes an asparaginase/glutaminase signature sequence spanning from amino acid 10 to amino acid 18 of SEQ ID NO:12.

Example 6

This Example describes the amplification and subsequent isolation of an asparaginase nucleic acid molecule from *D. immitis* female adult cDNA using primers designed for protein expression in pTrcHisB vector. This Example further discloses the production of a recombinant molecule and a recombinant cell of the present invention.

A DiASNase nucleic acid molecule was PCR amplified from female adult cDNA using a sense primer (DiASNase-XhoI) with the sequence, 5' CCGAGCTCGAGAATG-CAGTGTGAAGAAGCGCATGTTTTAG 3' (denoted herein as SEQ ID NO:23; XhoI site in bold) corresponding to a region of SEQ ID NO:25 spanning from nucleotide 69 through nucleotide 96, and including a linker sequence not found in SEQ ID NO:25. Also used was an antisense primer (DiASNase-HindIII) 5' CAGCCAAGCTTCTTACT-GAACTTTTTTCATCTTTTTTCATTCTAATGACTAG 3' (denoted herein as SEQ ID NO:24; HindIII site in bold) corresponding to a region of SEQ ID NO:25 spanning from nucleotide 1803 through nucleotide 1841, and also including a linker sequence not found in SEQ ID NO:25. PCR amplification of adult female cDNA with these primers yielded a 1770 bp product referred to herein as nDiASNase₁₇₇₀.

Nucleic acid molecule nDiASNase₁₇₇₀ was gel purified, cloned into a TA cloning vector (available from Invitrogen) and sequenced using an automated DNA sequencer. The sequence of the coding and complementary strands of nDiASNase₁₇₇₀ are herein represented by SEQ ID NO:11 and SEQ ID NO:13, respectively. Translation of SEQ ID NO:11 yields a protein of 590 amino acids, herein denoted PDiASNase₅₉₀, the amino acid sequence of which is presented in SEQ ID NO:12. Analysis of the amino acid sequence of *D. immitis* PDiASNase₅₉₀ (i.e., SEQ ID NO:12) predicts that PDiASNase₅₉₀ has an estimated molecular weight of about 66.2 kD and an estimated pI of about 5.96. The amino acid sequence of PDiASNase₅₉₀ has three potential N-glycosylation sites at positions 185, 192 and 297 of SEQ ID NO:12. In addition, there is an apparent asparaginase/glutaminase signature sequence found at residues 10–18 of SEQ ID NO:11.

Recombinant molecule PTrc-nDiASNase₁₇₇₀, containing from nucleotide 1 through nucleotide 1770 of nDiASNase₁₇₇₀, operatively linked to trc transcription control sequences and to a fusion sequence encoding a poly-histidine segment comprising 6 histidine residues, was produced in the following manner. Nucleic acid molecule nDiASNase₁₇₇₀ (containing nucleotides spanning from nucleotide 1 through nucleotide 1770 of SEQ ID NO:11) was PCR amplified as described above (using a sense primer (DiASNase-XhoI; SEQ ID NO:23) and an antisense primer (DiASNase-HindIII; SEQ ID NO:24). Recombinant molecule PTrc-nDiASNase₁₇₇₀ was produced by digesting nDiASNase₁₇₇₀ with XhoI and HindIII restriction endonucleases, gel purifying the resulting fragment, and directionally subcloning the fragment into expression vector

pTrcHisB (available from Invitrogen) that had been cleaved with XhoI and HindIII.

Recombinant molecule pTrc-nDiASNase₁₇₇₀ was transformed into *E. coli*, using standard techniques as disclosed in Sambrook et al., *ibid.*, to form recombinant cell *E. coli*:pTrc-nDiASNase₁₇₇₀.

Example 7

This Example describes the production of a DiASNase protein of the present invention in a prokaryotic cell, as well as studies to characterize that protein.

Recombinant cell *E. coli*:pTrc-nDiASNase₁₇₇₀, produced as described in Example 5, was cultured in shake-flasks containing an enriched bacterial growth medium and 0.1 mg/ml ampicillin at about 37° C. When the cells reached an OD₆₀₀ of about 0.5, expression of a *D. immitis* asparaginase protein was induced by addition of about 0.5 mM IPTG, followed by culture for about 3 hr at about 37° C. Protein production was monitored by SDS-PAGE of recombinant cell lysates, followed by Coomassie blue staining, using standard techniques. Recombinant cell *E. coli*:pTrc-nDiASNase₁₇₇₀ produced a fusion protein, denoted herein as PHis-PDiASNase₅₉₀, that migrated with an apparent molecular weight of about 66 kD.

Immunoblot analysis of recombinant cell *E. coli*:pTrc-nDiASNase₁₇₇₀ lysates indicated that an about 66 kD protein component of the cell lysates was able to bind a T₇ tag® monoclonal antibody (available from Novagen, Inc., Madison, Wis.) directed against the fusion portion of the recombinant PHis-PDiASNase₅₉₀ fusion protein. The PHis-PDiASNase₅₉₀ histidine fusion protein was separated from *E. coli* proteins in cell lysates by cobalt chelation chromatography with an imidazole gradient elution. Immunoblot analysis of the *E. coli*:pTrc-nDiASNase₁₇₇₀ lysates, column eluate and column void volume indicated that a 66 kD protein isolated from the *E. coli* lysates using cobalt column chromatography was able to selectively bind to the T₇ Tag® monoclonal antibody.

Example 8

This Example discloses the purification of a DiASNase fusion protein of the present invention from total cell lysates,

and the production of antibody directed against the purified DiASNase fusion protein.

DiASNase fusion protein PHis-PDiASNase₅₉₀, produced as described in Example 7, was separated from *E. coli* proteins by Talon™ Metal Affinity Resin Chromatography (available from CLONTECH Laboratories, Inc., Palo Alto, Calif.) according to the manufacturer's instructions. The PHis-PDiASNase₅₉₀ fusion protein was eluted using an imidazole gradient, pooled and dialyzed against 1 X PBS to produce cobalt column-purified PHis-PDiASNase₅₉₀. The dialyzed protein was then concentrated using a 10K molecular weight cut off Centrifugal Ultra-free® concentrator (available from Millipore Corporation, Bedford, Mass.). The protein content of the fusion protein was determined by using a MicroBCA™ Protein Assay (available from Pierce, Rockford, Ill.). The purified protein was tested for its purity by SDS PAGE and immunoblot analysis.

Anti-PHis-PDiASNase₅₉₀ (anti-DiASNase) antisera was produced as follows: A rabbit was immunized subcutaneously, first with approximately 75 µg of the purified PHis-PDiASNase₅₉₀ protein with complete Freund's Adjuvant, and then with three subsequent immunizations of the same dose of the fusion protein mixed in Incomplete Freund's Adjuvant. Bleeding and immunization were performed at alternate weeks. Sera were separated and stored at -70° C. until use.

The immunoglobulin G (IgG) fraction from rabbit anti-DiASNase antisera (anti-DiASNase-IgG fraction) was collected by 50% ammonium sulfate precipitation. Ammonium ions were removed by extensive dialysis in 0.1M PBS, pH 7.2. The IgG content was determined by measuring absorbance at OD₂₈₀ as compared with a blank PBS control. The anti-DiASNase-IgG fraction had a titer of 1:512,000 as determined by ELISA.

While various embodiments of the present invention have been described in detail, it is apparent that modifications and adaptations of those embodiments will occur to those skilled in the art. It is to be expressly understood, however, that such modifications and adaptations are within the scope of the present invention, as set forth in the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 26

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1753 nucleotides

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i x) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..1518

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAT TCA TCA GAT ATG ACA TTT GAT GAC TGG ATT CAT ATC GGT

-continued

Asp 1	Ser	Ser	Asp	Met 5	Thr	Phe	Asp	Asp	Trp 10	Ile	His	Ile	Gly	
AAA Lys 15	GAT Asp	ATT Ile	CAA Gln	AGA Arg	GCT Ala 20	TAC Tyr	GAT Asp	CAA Gln	TAT Tyr	GTG Val 25	GGC Gly	TTT Phe	GTT Val	84
ATA Ile 30	TTA Leu	CAT His	GGT Gly	ACT Thr	GAT Asp	ACA Thr 35	CTG Leu	GCA Ala	TAT Tyr	ACG Thr 40	GCA Ala	TGT Cys	GCT Ala	126
TTG Leu	TCA Ser	TTT Phe 45	ATG Met	CTG Leu	GAG Glu	AAC Asn 50	GTA Val	AGA Arg	AAA Lys	CCC Pro	ATT Ile	GTT Val 55	ATT Ile	168
ACA Thr	GGA Gly	GCT Ala	CAA Gln 60	ATA Ile	CCA Pro	GTG Val	TGT Cys	GAA Glu 65	GTT Val	CGT Arg	TCT Ser	GAC Asp	GGT Gly 70	210
CGA Arg	GAA Glu	AAT Asn	TTG Leu	ATT Ile 75	GGT Gly	GCA Ala	CTG Leu	ATT Ile	ATT Ile 80	GCA Ala	GCC Ala	AAT Asn	TAT Tyr	252
GAT Asp 85	ATT Ile	CCT Pro	GAA Glu	GTT Val 90	ACT Thr	GTA Val	TAT Tyr	TTC Phe	AAT Asn	AAT Asn 95	AAG Lys	CTG Leu	TTT Phe	294
CGA Arg 100	GGA Gly	AAT Asn	CGT Arg	ACA Thr	GTA Val	AAA Lys 105	ATA Ile	GAT Asp	AAC Asn	AGA Arg	TCA Ser 110	ATG Met	GAT Asp	336
GCT Ala	TTT Phe	GAA Glu 115	AGT Ser	CCA Pro	AAT Asn	ATG Met 120	CTT Leu	CCA Pro	ATT Ile	GCT Ala	TAC Tyr	ATG Met 125	GAT Asp	378
GTT Val	GAT Asp	ATA Ile	AAA Lys 130	GTT Val	AAT Asn	TAT Tyr	GAT Asp	TCA Ser 135	ATA Ile	TTT Phe	CGT Arg	TCA Ser	CCA Pro 140	420
TCA Ser	ATG Met	GCT Ala	CCA Pro	TTC Phe 145	GTA Val	GTA Val	CAC His	GAC Asp	CAA Gln 150	TTA Leu	TGT Cys	CGA Arg	AAT Asn	462
GTT Val 155	GGA Gly	TTG Leu	TTG Leu	AGA Arg	ATT Ile 160	TTT Phe	CCA Pro	TCG Ser	ATG Met	TCT Ser 165	ATA Ile	GAA Glu	AAC Asn	504
GTT Val 170	AGA Arg	GCA Ala	TCC Ser	TTG Leu	CAG Gln	GCA Ala 175	CCT Pro	ATT Ile	GAA Glu	GGT Gly	GTT Val 180	GTT Val	CTG Leu	546
CAG Gln	ACG Thr	TTT Phe 185	GGT Gly	GCT Ala	GGT Gly	AAT Asn 190	ATG Met	CCC Pro	TCC Ser	CAT His	AGG Arg	ACA Thr 195	GAT Asp	588
ATA Ile	ATC Ile	GAT Asp	GAA Glu 200	TTG Leu	AAA Lys	AAA Lys	GCT Ala	GTT Val 205	GAT Asp	CGA Arg	GGA Gly	TGT Cys	ATT Ile 210	630
ATT Ile	ATT Ile	AAT Asn	TGC Cys	TCA Ser 215	CAG Gln	TGT Cys	GTC Val	CGT Arg	GGA Gly 220	CAA Gln	GTA Val	GAT Asp	ATT Ile	672
CAT His 225	TAT Tyr	TTA Leu	ACG Thr	GGA Gly	AAG Lys 230	GTT Val	CTA Leu	TAC Tyr	GAC Asp	ATG Met 235	GGA Gly	ATT Ile	ATT Ile	714
CCT Pro 240	GGT Gly	TCA Ser	GAT Asp	ATG Met	ACT Thr	GCA Ala 245	GAA Glu	GCA Ala	GCA Ala	TTA Leu 250	ACA Thr	AAA Lys	TTA Leu	756
TCG Ser	TAT Tyr	GTA Val 255	TTG Leu	AGC Ser	AAA Lys	GAT Asp	TGT Cys 260	TGG Trp	GAA Glu	CTT Leu	GTG Val	GAG Glu 265	AAA Lys	798
AAA Lys	GCA Ala	ATG Met	ATG Met 270	GTT Val	AAA Lys	AAT Asn	ATC Ile 275	AGA Arg	GGC Gly	GAA Glu	TTA Leu	ACT Thr	GTT Val 280	840
GCA	AAA	GCA	GAA	CCA	CTC	AAA	GAT	CTA	GAA	ATC	GTA	TCA	CAG	882

-continued

Ala	Lys	Ala	Glu	Pro	Leu	Lys	Asp	Leu	Glu	Ile	Val	Ser	Gln	
				285					290					
ATG	GCA	AGA	TTC	CTG	CAT	CTA	AGT	TCT	TCT	CAT	GAA	ATG	AAA	924
Met	Ala	Arg	Phe	Leu	His	Leu	Ser	Ser	Ser	His	Glu	Met	Lys	
295					300					305				
CTC	CTC	TGT	CAT	GCT	ATT	TTT	CCA	CAA	TTA	TTG	TGT	TAT	GCA	966
Leu	Leu	Cys	His	Ala	Ile	Phe	Pro	Gln	Leu	Leu	Cys	Tyr	Ala	
	310					315					320			
GCT	AGT	AAT	GGG	GAT	ATC	GAA	ATG	CTA	AAG	GCA	CTT	CAT	GAA	1008
Ala	Ser	Asn	Gly	Asp	Ile	Glu	Met	Leu	Lys	Ala	Leu	His	Glu	
		325					330					335		
AAT	GGA	GTT	GAT	CTT	TCG	GTT	GTT	GAC	TAT	AAT	GGA	CGC	AAT	1050
Asn	Gly	Val	Asp	Leu	Ser	Val	Val	Asp	Tyr	Asn	Gly	Arg	Asn	
			340					345					350	
GCT	TTG	CAT	GTA	GCA	GCG	AGT	GCA	GGT	CAC	GTT	GGT	GCT	GTC	1092
Ala	Leu	His	Val	Ala	Ala	Ser	Ala	Gly	His	Val	Gly	Ala	Val	
				355					360					
AAA	TAT	CTG	TTG	ACC	CAA	GGT	GTT	AGT	TTT	CAT	CTG	AGA	GAT	1134
Lys	Tyr	Leu	Leu	Thr	Gln	Gly	Val	Ser	Phe	His	Leu	Arg	Asp	
365					370					375				
CAA	TGG	GAT	GAG	AAT	GCC	CTC	GTA	AGT	GCA	GTA	AAA	ATG	AAA	1176
Gln	Trp	Asp	Glu	Asn	Ala	Leu	Val	Ser	Ala	Val	Lys	Met	Lys	
	380					385					390			
AAT	AAG	ATC	TTA	ATT	GAA	ACT	TTG	CGA	TCT	GCA	GGG	GCA	CTG	1218
Asn	Lys	Ile	Leu	Ile	Glu	Thr	Leu	Arg	Ser	Ala	Gly	Ala	Leu	
		395					400					405		
CTT	TCC	ATA	AAT	TCA	CGC	AGA	TTA	GGT	GTT	GAA	CTA	TGT	CTA	1260
Leu	Ser	Ile	Asn	Ser	Arg	Arg	Leu	Gly	Val	Glu	Leu	Cys	Leu	
			410					415					420	
TGT	GCC	AGC	TAT	GGC	GAC	ACG	GAA	ACA	CTG	AAT	TCT	TGG	CTT	1302
Cys	Ala	Ser	Tyr	Gly	Asp	Thr	Glu	Thr	Leu	Asn	Ser	Trp	Leu	
				425					430					
GCT	GCC	GGA	GCC	GAT	ATA	AAT	CAA	CAA	GAT	TAC	AAT	GGC	GAA	1344
Ala	Ala	Gly	Ala	Asp	Ile	Asn	Gln	Gln	Asp	Tyr	Asn	Gly	Glu	
435					440					445				
ACT	GCT	TTG	CAT	ATT	GCG	GTG	AAA	TCG	AGA	AAT	AAG	CAA	TTG	1386
Thr	Ala	Leu	His	Ile	Ala	Val	Lys	Ser	Arg	Asn	Lys	Gln	Leu	
	450					455					460			
GTA	CAT	TAT	TTG	CTG	GAT	AGA	GAT	GCA	GAT	CCA	TAC	AAA	ATT	1428
Val	His	Tyr	Leu	Leu	Asp	Arg	Asp	Ala	Asp	Pro	Tyr	Lys	Ile	
		465				470						475		
GAC	GAT	TTT	AAT	TTA	ACG	CCT	CTT	AGA	CAT	GCT	AAA	AAA	CTT	1470
Asp	Asp	Phe	Asn	Leu	Thr	Pro	Leu	Arg	His	Ala	Lys	Lys	Leu	
			480					485					490	
AAT	TTA	CAA	GAT	CTA	GTC	ATT	AGA	ATG	AAA	AAG	ATG	AAA	AAA	1512
Asn	Leu	Gln	Asp	Leu	Val	Ile	Arg	Met	Lys	Lys	Met	Lys	Lys	
				495				500						
GTT	CAG	TAA	TGTTGCTGCA	GAAAATAAAG	ATCTTATGCA	CTCAGAATGT								1561
Val	Gln													
505														
ATTCAGAAGT	ATGGTACAAA	AGCCTTAAAT	TATGCTAGAT	CTTGCAATGAT										1611
TTCTAGCTTT	TTAAATGGTA	ATTTTTGTTC	CGTCTTTTTT	CGCAAAGACT										1661
GATATAATTT	AATGAAAAAA	AACCTTGTTT	ATTCATCGAT	TCCTTTTTTA										1711
AACAAAATAG	TATTTAATGG	CTAAAAAAA	AAAAAAA	AA										1753

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 506 amino acids

-continued

(B) TYPE: amino acid
 (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:2:

A s p 1	S e r	S e r	A s p	M e t 5	T h r	P h e	A s p	A s p	T r p 10	I l e	H i s	I l e	G l y
L y s 15	A s p	I l e	G l n	A r g	A l a 20	T y r	A s p	G l n	T y r	V a l 25	G l y	P h e	V a l
I l e 30	L e u	H i s	G l y	T h r	A s p	T h r 35	L e u	A l a	T y r	T h r	A l a 40	C y s	A l a
L e u	S e r	P h e 45	M e t	L e u	G l u	A s n	V a l 50	A r g	L y s	P r o	I l e	V a l 55	I l e
T h r	G l y	A l a	G l n 60	I l e	P r o	V a l	C y s	G l u 65	V a l	A r g	S e r	A s p	G l y 70
A r g	G l u	A s n	L e u	I l e 75	G l y	A l a	L e u	I l e	I l e 80	A l a	A l a	A s n	T y r
A s p 85	I l e	P r o	G l u	V a l	T h r 90	V a l	T y r	P h e	A s n	A s n 95	L y s	L e u	P h e
A r g	G l y 100	A s n	A r g	T h r	V a l	L y s 105	I l e	A s p	A s n	A r g	S e r 110	M e t	A s p
A l a	P h e	G l u 115	S e r	P r o	A s n	M e t 120	L e u	P r o	I l e	A l a	T y r	M e t 125	A s p
V a l	A s p	I l e	L y s 130	V a l	A s n	T y r	A s p	S e r 135	I l e	P h e	A r g	S e r	P r o 140
S e r	M e t	A l a	P r o	P h e 145	V a l	V a l	H i s	A s p	G l n 150	L e u	C y s	A r g	A s n
V a l 155	G l y	L e u	L e u	A r g	I l e 160	P h e	P r o	S e r	M e t 165	S e r	I l e	G l u	A s n
V a l 170	A r g	A l a	S e r	L e u	G l n	A l a 175	P r o	I l e	G l u	G l y	V a l 180	V a l	L e u
G l n	T h r	P h e 185	G l y	A l a	G l y	A s n	M e t 190	P r o	S e r	H i s	A r g	T h r 195	A s p
I l e	I l e	A s p	G l u 200	L e u	L y s	L y s	A l a	V a l 205	A s p	A r g	G l y	C y s	I l e 210
I l e	I l e	A s n	C y s 215	S e r	G l n	C y s	V a l	A r g	G l y 220	G l n	V a l	A s p	I l e
H i s 225	T y r	L e u	T h r	G l y	L y s 230	V a l	L e u	T y r	A s p	M e t 235	G l y	I l e	I l e
P r o	G l y 240	S e r	A s p	M e t	T h r	A l a 245	G l u	A l a	A l a	L e u	T h r 250	L y s	L e u
S e r	T y r	V a l 255	L e u	S e r	L y s	A s p	C y s 260	T r p	G l u	L e u	V a l	G l u 265	L y s
L y s	A l a	M e t	M e t 270	V a l	L y s	A s n	I l e	A r g 275	G l y	G l u	L e u	T h r	V a l 280
A l a	L y s	A l a	G l u	P r o 285	L e u	L y s	A s p	L e u	G l u 290	I l e	V a l	S e r	G l n
M e t 295	A l a	A r g	P h e	L e u	H i s 300	L e u	S e r	S e r	S e r	H i s 305	G l u	M e t	L y s
L e u 310	L e u	C y s	H i s	A l a	I l e	P h e 315	P r o	G l n	L e u	L e u	C y s 320	T y r	A l a
A l a	S e r	A s n 325	G l y	A s p	I l e	G l u	M e t 330	L e u	L y s	A l a	L e u	H i s 335	G l u
A s n	G l y	V a l	A s p	L e u	S e r	V a l	V a l	A s p	T y r	A s n	G l y	A r g	A s n

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			3 4 0					3 4 5				3 5 0	
A l a	L e u	H i s	V a l	A l a	A l a	S e r	A l a	G l y	H i s	V a l	G l y	A l a	V a l
				3 5 5					3 6 0				
L y s	T y r	L e u	L e u	T h r	G l n	G l y	V a l	S e r	P h e	H i s	L e u	A r g	A s p
3 6 5					3 7 0					3 7 5			
G l n	T r p	A s p	G l u	A s n	A l a	L e u	V a l	S e r	A l a	V a l	L y s	M e t	L y s
	3 8 0					3 8 5					3 9 0		
A s n	L y s	I l e	L e u	I l e	G l u	T h r	L e u	A r g	S e r	A l a	G l y	A l a	L e u
		3 9 5					4 0 0					4 0 5	
L e u	S e r	I l e	A s n	S e r	A r g	A r g	L e u	G l y	V a l	G l u	L e u	C y s	L e u
			4 1 0					4 1 5					4 2 0
C y s	A l a	S e r	T y r	G l y	A s p	T h r	G l u	T h r	L e u	A s n	S e r	T r p	L e u
				4 2 5					4 3 0				
A l a	A l a	G l y	A l a	A s p	I l e	A s n	G l n	G l n	A s p	T y r	A s n	G l y	G l u
4 3 5					4 4 0					4 4 5			
T h r	A l a	L e u	H i s	I l e	A l a	V a l	L y s	S e r	A r g	A s n	L y s	G l n	L e u
	4 5 0					4 5 5					4 6 0		
V a l	H i s	T y r	L e u	L e u	A s p	A r g	A s p	A l a	A s p	P r o	T y r	L y s	I l e
		4 6 5					4 7 0					4 7 5	
A s p	A s p	P h e	A s n	L e u	T h r	P r o	L e u	A r g	H i s	A l a	L y s	L y s	L e u
			4 8 0					4 8 5					4 9 0
A s n	L e u	G l n	A s p	L e u	V a l	I l e	A r g	M e t	L y s	L y s	M e t	L y s	L y s
				4 9 5					5 0 0				
V a l	G l n												
5 0 5													

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1753 nucleotides
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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TTTTTTTTTT TTTTTTTTTT AGCCATTAAA TACTATTTTG TTTAAAAAAG      5 0
GAATCGATGA ATAAACAAGG TTTTTTTTCA TTAAATTATA TCAGTCTTTG      1 0 0
CGAAAAAAGA CGGAACAAAA ATTACCATTT AAAAAAGCTAG AAATCATGCA      1 5 0
AGATCTAGCA TAATTTAAGG CTTTTGTACC ATACTTCTGA ATACATTCTG      2 0 0
AGTGCATAAG ATCTTTATTT TCTGCAGCAA CATTACTGAA CTTTTTTCAT      2 5 0
CTTTTTTCATT CTAATGACTA GATCTTGTA AATTAAGTTTT TTAGCATGTC      3 0 0
TAAGAGGCGT TAAATTA AAA TCGTCAATTT TGTATGGATC TGCATCTCTA      3 5 0
TCCAGCAAAT AATGTACCAA TTGCTTATTT CTCGATTTCA CCGCAATATG      4 0 0
CAAAGCAGTT TCGCCATTGT AATCTTGTTG ATTTATATCG GCTCCGGCAG      4 5 0
CAAGCCAAGA ATTCAGTGTT TCCGTGTCGC CATAGCTGGC ACATAGACAT      5 0 0
AGTTCAACAC CTAATCTGCG TGAATTTATG GAAAGCAGTG CCCCTGCAGA      5 5 0
TCGCAAAGTT TCAATTAAGA TCTTATTTTT CTTTTTACT GCACTTACGA      6 0 0
GGGCATTCTC ATCCCATTTGA TCTCTCAGAT GAAAAC TAAC ACCTTGGGTC      6 5 0
AACAGATATT TGACAGCACC AACGTGACCT GCACTCGCTG CTACATGCAA      7 0 0
AGCATTGCGT CCATTATAGT CAACAACCGA AAGATCAACT CCATTTTTCAT      7 5 0

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GAAGTGCCTT	TAGCATTTCG	ATATCCCCAT	TACTAGCTGC	ATAACACAAT	800
AATTGTGGAA	AAATAGCATG	ACAGAGGAGT	TTCATTTCAT	GAGAAGAACT	850
TAGATGCAGG	AATCTTGCCA	TCTGTGATAC	GATTTCTAGA	TCTTTGAGTG	900
GTTCTGCTTT	TGCAACAGTT	AATTCGCCTC	TGATATTTTT	AACCATCATT	950
GCTTTTTTCT	CCACAAGTTC	CCAACAATCT	TTGCTCAATA	CATACGATAA	1000
TTTTGTAAAT	GCTGCTTCTG	CAGTCATATC	TGAACCAGGA	ATAATTCCCA	1050
TGTCGTATAG	AACCTTTCCC	GTTAAATAAT	GAATATCTAC	TTGTCCACGG	1100
ACACACTGTG	AGCAATTAAT	AATAATACAT	CCTCGATCAA	CAGCTTTTTT	1150
CAATTCATCG	ATTATATCTG	TCCTATGGGA	GGGCATATTA	CCAGCACCAA	1200
ACGTCTGCAG	AACAACACCT	TCAATAGGTG	CCTGCAAGGA	TGCTCTAACG	1250
TTTTCTATAG	ACATCGATGG	AAAAATTCTC	AACAATCCAA	CATTTGACA	1300
TAATTGGTCG	TGTACTACGA	ATGGAGCCAT	TGATGGTGAA	CGAAATATTG	1350
AATCATAATT	AACTTTTATA	TCAACATCCA	TGTAAGCAAT	TGGAAGCATA	1400
TTTGGACTTT	CAAAGCATC	CATTGATCTG	TTATCTATTT	TTACTGTACG	1450
ATTTCCCTCGA	AACAGCTTAT	TATTGAAATA	TACAGTAACT	TCAGGAATAT	1500
CATAATTGGC	TGCAATAATC	AGTGCACCAA	TCAAATTTTC	TCGACCGTCA	1550
GAACGAACTT	CACACACTGG	TATTTGAGCT	CCTGTAATAA	CAATGGGTTT	1600
TCTTACGTTC	TCCAGCATAA	ATGACAAAGC	ACATGCCGTA	TATGCCAGTG	1650
TATCAGTACC	ATGTAATATA	ACAAAGCCCA	CATATTGATC	GTAAGCTCTT	1700
TGAATATCTT	TACCGATATG	AATCCAGTCA	TCAAATGTCA	TATCTGATGA	1750
ATC					1753

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1518 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

- (i x) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..1518

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GAT	TCA	TCA	GAT	ATG	ACA	TTT	GAT	GAC	TGG	ATT	CAT	ATC	GGT	42
Asp	Ser	Ser	Asp	Met	Thr	Phe	Asp	Asp	Trp	Ile	His	Ile	Gly	
	1			5					10					
AAA	GAT	ATT	CAA	AGA	GCT	TAC	GAT	CAA	TAT	GTG	GGC	TTT	GTT	84
Lys	Asp	Ile	Gln	Arg	Ala	Tyr	Asp	Gln	Tyr	Val	Gly	Phe	Val	
	15			20					25					
ATA	TTA	CAT	GGT	ACT	GAT	ACA	CTG	GCA	TAT	ACG	GCA	TGT	GCT	126
Ile	Leu	His	Gly	Thr	Asp	Thr	Leu	Ala	Tyr	Thr	Ala	Cys	Ala	
	30			35					40					
TTG	TCA	TTT	ATG	CTG	GAG	AAC	GTA	AGA	AAA	CCC	ATT	GTT	ATT	168
Leu	Ser	Phe	Met	Leu	Glu	Asn	Val	Arg	Lys	Pro	Ile	Val	Ile	
		45				50					55			
ACA	GGA	GCT	CAA	ATA	CCA	GTG	TGT	GAA	GTT	CGT	TCT	GAC	GGT	210
Thr	Gly	Ala	Gln	Ile	Pro	Val	Cys	Glu	Val	Arg	Ser	Asp	Gly	
			60					65					70	

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CGA Arg	GAA Glu	AAT Asn	TTG Leu	ATT Ile 75	GGT Gly	GCA Ala	CTG Leu	ATT Ile	ATT Ile 80	GCA Ala	GCC Ala	AAT Asn	TAT Tyr	252
GAT Asp 85	ATT Ile	CCT Pro	GAA Glu	GTT Val	ACT Thr 90	GTA Val	TAT Tyr	TTC Phe	AAT Asn	AAT Asn 95	AAG Lys	CTG Leu	TTT Phe	294
CGA Arg 100	GGA Gly	AAT Asn	CGT Arg	ACA Thr	GTA Val	AAA Lys 105	ATA Ile	GAT Asp	AAC Asn	AGA Arg	TCA Ser 110	ATG Met	GAT Asp	336
GCT Ala	TTT Phe	GAA Glu 115	AGT Ser	CCA Pro	AAT Asn	ATG Met	CTT Leu 120	CCA Pro	ATT Ile	GCT Ala	TAC Tyr	ATG Met 125	GAT Asp	378
GTT Val	GAT Asp	ATA Ile	AAA Lys 130	GTT Val	AAT Asn	TAT Tyr	GAT Asp	TCA Ser 135	ATA Ile	TTT Phe	CGT Arg	TCA Ser	CCA Pro 140	420
TCA Ser	ATG Met	GCT Ala	CCA Pro	TTC Phe 145	GTA Val	GTA Val	CAC His	GAC Asp	CAA Gln 150	TTA Leu	TGT Cys	CGA Arg	AAT Asn	462
GTT Val 155	GGA Gly	TTG Leu	TTG Leu	AGA Arg	ATT Ile 160	TTT Phe	CCA Pro	TCG Ser	ATG Met	TCT Ser 165	ATA Ile	GAA Glu	AAC Asn	504
GTT Val 170	AGA Arg	GCA Ala	TCC Ser	TTG Leu	CAG Gln	GCA Ala 175	CCT Pro	ATT Ile	GAA Glu	GGT Gly	GTT Val 180	GTT Val	CTG Leu	546
CAG Gln	ACG Thr	TTT Phe 185	GGT Gly	GCT Ala	GGT Gly	AAT Asn	ATG Met 190	CCC Pro	TCC Ser	CAT His	AGG Arg	ACA Thr 195	GAT Asp	588
ATA Ile	ATC Ile	GAT Asp	GAA Glu 200	TTG Leu	AAA Lys	AAA Lys	GCT Ala 205	GTT Val	GAT Asp	CGA Arg	GGA Gly	TGT Cys	ATT Ile 210	630
ATT Ile	ATT Ile	AAT Asn	TGC Cys	TCA Ser 215	CAG Gln	TGT Cys	GTC Val	CGT Arg	GGA Gly 220	CAA Gln	GTA Val	GAT Asp	ATT Ile	672
CAT His 225	TAT Tyr	TTA Leu	ACG Thr	GGA Gly	AAG Lys 230	GTT Val	CTA Leu	TAC Tyr	GAC Asp	ATG Met 235	GGA Gly	ATT Ile	ATT Ile	714
CCT Pro 240	GGT Gly	TCA Ser	GAT Asp	ATG Met	ACT Thr	GCA Ala 245	GAA Glu	GCA Ala	GCA Ala	TTA Leu	ACA Thr 250	AAA Lys	TTA Leu	756
TCG Ser	TAT Tyr	GTA Val 255	TTG Leu	AGC Ser	AAA Lys	GAT Asp	TGT Cys 260	TGG Trp	GAA Glu	CTT Leu	GTG Val 265	GAG Glu	AAA Lys	798
AAA Lys	GCA Ala	ATG Met	ATG Met 270	GTT Val	AAA Lys	AAT Asn	ATC Ile 275	AGA Arg	GGC Gly	GAA Glu	TTA Leu	ACT Thr 280	GTT Val	840
GCA Ala	AAA Lys	GCA Ala	GAA Glu	CCA Pro 285	CTC Leu	AAA Lys	GAT Asp	CTA Leu	GAA Glu 290	ATC Ile	GTA Val	TCA Ser	CAG Gln	882
ATG Met 295	GCA Ala	AGA Arg	TTC Phe	CTG Leu	CAT His 300	CTA Leu	AGT Ser	TCT Ser	TCT Ser	CAT His 305	GAA Glu	ATG Met	AAA Lys	924
CTC Leu 310	CTC Leu	TGT Cys	CAT His	GCT Ala	ATT Ile	TTT Phe 315	CCA Pro	CAA Gln	TTA Leu	TTG Leu 320	TGT Cys	TAT Tyr	GCA Ala	966
GCT Ala	AGT Ser	AAT Asn 325	GGG Gly	GAT Asp	ATC Ile	GAA Glu 330	ATG Met 330	CTA Leu	AAG Lys	GCA Ala	CTT Leu	CAT His 335	GAA Glu	1008
AAT Asn	GGA Gly	GTT Val	GAT Asp 340	CTT Leu	TCG Ser	GTT Val	GTT Val	GAC Asp 345	TAT Tyr	AAT Asn	GGA Gly	CGC Arg	AAT Asn 350	1050

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GCT	TTG	CAT	GTA	GCA	GCG	AGT	GCA	GGT	CAC	GTT	GGT	GCT	GTC	1092
Ala	Leu	His	Val	Ala	Ala	Ser	Ala	Gly	His	Val	Gly	Ala	Val	
				355					360					
AAA	TAT	CTG	TTG	ACC	CAA	GGT	GTT	AGT	TTT	CAT	CTG	AGA	GAT	1134
Lys	Tyr	Leu	Leu	Thr	Gln	Gly	Val	Ser	Phe	His	Leu	Arg	Asp	
365					370					375				
CAA	TGG	GAT	GAG	AAT	GCC	CTC	GTA	AGT	GCA	GTA	AAA	ATG	AAA	1176
Gln	Trp	Asp	Glu	Asn	Ala	Leu	Val	Ser	Ala	Val	Lys	Met	Lys	
	380					385					390			
AAT	AAG	ATC	TTA	ATT	GAA	ACT	TTG	CGA	TCT	GCA	GGG	GCA	CTG	1218
Asn	Lys	Ile	Leu	Ile	Glu	Thr	Leu	Arg	Ser	Ala	Gly	Ala	Leu	
		395				400						405		
CTT	TCC	ATA	AAT	TCA	CGC	AGA	TTA	GGT	GTT	GAA	CTA	TGT	CTA	1260
Leu	Ser	Ile	Asn	Ser	Arg	Arg	Leu	Gly	Val	Glu	Leu	Cys	Leu	
			410					415					420	
TGT	GCC	AGC	TAT	GGC	GAC	ACG	GAA	ACA	CTG	AAT	TCT	TGG	CTT	1302
Cys	Ala	Ser	Tyr	Gly	Asp	Thr	Glu	Thr	Leu	Asn	Ser	Trp	Leu	
				425					430					
GCT	GCC	GGA	GCC	GAT	ATA	AAT	CAA	CAA	GAT	TAC	AAT	GGC	GAA	1344
Ala	Ala	Gly	Ala	Asp	Ile	Asn	Gln	Gln	Asp	Tyr	Asn	Gly	Glu	
435					440					445				
ACT	GCT	TTG	CAT	ATT	GCG	GTG	AAA	TCG	AGA	AAT	AAG	CAA	TTG	1386
Thr	Ala	Leu	His	Ile	Ala	Val	Lys	Ser	Arg	Asn	Lys	Gln	Leu	
	450					455					460			
GTA	CAT	TAT	TTG	CTG	GAT	AGA	GAT	GCA	GAT	CCA	TAC	AAA	ATT	1428
Val	His	Tyr	Leu	Leu	Asp	Arg	Asp	Ala	Asp	Pro	Tyr	Lys	Ile	
		465				470						475		
GAC	GAT	TTT	AAT	TTA	ACG	CCT	CTT	AGA	CAT	GCT	AAA	AAA	CTT	1470
Asp	Asp	Phe	Asn	Leu	Thr	Pro	Leu	Arg	His	Ala	Lys	Lys	Leu	
			480					485					490	
AAT	TTA	CAA	GAT	CTA	GTC	ATT	AGA	ATG	AAA	AAG	ATG	AAA	AAA	1512
Asn	Leu	Gln	Asp	Leu	Val	Ile	Arg	Met	Lys	Lys	Met	Lys	Lys	
				495					500					
GTT	CAG													1518
Val	Gln													
505														

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1518 nucleotides
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTGAACTTTT	TTCATCTTTT	TCATTCTAAT	GACTAGATCT	TGTAAATTAA	50
GTTTTTTAGC	ATGTCTAAGA	GGCGTTAAAT	TAAAATCGTC	AATTTTGTAT	100
GGATCTGCAT	CTCTATCCAG	CAAATAATGT	ACCAATTGCT	TATTTCTCGA	150
TTTCACCGCA	ATATGCAAAG	CAGTTTCGCC	ATTGTAATCT	TGTTGATTTA	200
TATCGGCTCC	GGCAGCAAGC	CAAGAATTCA	GTGTTTCCGT	GTCGCCATAG	250
CTGGCACATA	GACATAGTTC	AACACCTAAT	CTGCGTGAAT	TTATGGAAAG	300
CAGTGCCCCT	GCAGATCGCA	AAGTTTCAAT	TAAGATCTTA	TTTTTCATTT	350
TTACTGCACT	TACGAGGGCA	TTCTCATCCC	ATTGATCTCT	CAGATGAAAA	400
CTAACACCTT	GGGTCAACAG	ATATTTGACA	GCACCAACGT	GACCTGCACT	450
CGCTGCTACA	TGCAAAGCAT	TGCGTCCATT	ATAGTCAACA	ACCGAAAGAT	500

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CAACTCCATT	TTCATGAAGT	GCCTTTAGCA	TTTCGATATC	CCCATTACTA	5 5 0
GCTGCATAAC	ACAATAATTG	TGGAAAAATA	GCATGACAGA	GGAGTTTCAT	6 0 0
TTCATGAGAA	GAAC TTAGAT	GCAGGAATCT	TGCCATCTGT	GATACGATTT	6 5 0
CTAGATCTTT	GAGTGGTTCT	GCTTTTGCAA	CAGTTAATTC	GCCTCTGATA	7 0 0
TTTTTAACCA	TCATTGCTTT	TTTCTCCACA	AGTTCCCAAC	AATCTTTGCT	7 5 0
CAATACATAC	GATAATTTTG	TTAATGCTGC	TTCTGCAGTC	ATATCTGAAC	8 0 0
CAGGAATAAT	TCCCATGTCTG	TATAGAACCT	TTCCCGTTAA	ATAATGAATA	8 5 0
TCTACTTGTC	CACGGACACA	CTGTGAGCAA	TTAATAATAA	TACATCCTCG	9 0 0
ATCAACAGCT	TTTTTCAATT	CATCGATTAT	ATCTGTCCTA	TGGGAGGGCA	9 5 0
TATTACCAGC	ACCAAACGTC	TGCAGAACAA	CACCTTCAAT	AGGTGCCTGC	1 0 0 0
AAGGATGCTC	TAACGTTTTTC	TATAGACATC	GATGGAAAAA	TTCTCAACAA	1 0 5 0
TCCAACATTT	CGACATAATT	GGTCGTGTAC	TACGAATGGA	GCCATTGATG	1 1 0 0
GTGAACGAAA	TATTGAATCA	TAATTAACTT	TTATATCAAC	ATCCATGTAA	1 1 5 0
GCAATTGGAA	GCATATTTGG	ACTTTCAAAA	GCATCCATTG	ATCTGTTATC	1 2 0 0
TATTTTTACT	GTACGATTTTC	CTCGAAACAG	CTTATTATTG	AAATATACAG	1 2 5 0
TAAC TTCAGG	AATATCATAA	TTGGCTGCAA	TAATCAGTGC	ACCAATCAAA	1 3 0 0
TTTTCTCGAC	CGTCAGAACG	AACTTCACAC	ACTGGTATTT	GAGCTCCTGT	1 3 5 0
AATAACAATG	GGTTTTCTTA	CGTTCTCCAG	CATAAATGAC	AAAGCACATG	1 4 0 0
CCGTATATGC	CAGTGTATCA	GTACCATGTA	ATATAACAAA	GCCCACATAT	1 4 5 0
TGATCGTAAG	CTCTTTGAAT	ATCTTTACCG	ATATGAATCC	AGTCATCAAA	1 5 0 0
TGTCATATCT	GATGAATC				1 5 1 8

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 439 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

- (i x) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 69..437

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGTTTAATTA	CCCAAGTTTG	AGCAATTAAA	TTAGATTGGA	AGTATTTATA	5 0									
CAAATATCAT	TCAGTCCG	ATG	CAG	TGT	GAA	GAA	GCG	CAT	GTT	TTA	9 5			
		Met	Gln	Cys	Glu	Glu	Ala	His	Val	Leu				
		1				5								
GTG	CTA	TAT	ACA	GGT	GGA	ACG	ATT	GGG	ATG	AAA	TAC	ATT	GAT	1 3 7
Val	Leu	Tyr	Thr	Gly	Gly	Thr	Ile	Gly	Met	Lys	Tyr	Ile	Asp	
1 0				1 5						2 0				
GGA	GTG	TAT	CAG	CCA	GAA	GCT	AAT	TAT	CTT	CTG	CAT	GCC	ATA	1 7 9
Gly	Val	Tyr	Gln	Pro	Glu	Ala	Asn	Tyr	Leu	Leu	His	Ala	Ile	
	2 5					3 0					3 5			
CGT	GAT	TTA	TCA	CTA	TTA	AAC	GAT	GAT	GAT	TAT	GTG	TCC	ACA	2 2 1
Arg	Asp	Leu	Ser	Leu	Leu	Asn	Asp	Asp	Asp	Tyr	Val	Ser	Thr	
		4 0					4 5					5 0		
TAT	TAT	TCT	GAC	GCC	GAA	ATA	AGG	CCA	TAT	TGT	TTG	CCA	CCA	2 6 3
Tyr	Tyr	Ser	Asp	Ala	Glu	Ile	Arg	Pro	Tyr	Cys	Leu	Pro	Pro	

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55			60			65								
CTA	CAA	CAT	TCA	AAA	AAA	CGT	GTT	GTT	TAT	TGG	ATG	ATC	GAA	305
Leu	Gln	His	Ser	Lys	Lys	Arg	Val	Val	Tyr	Trp	Met	Ile	Glu	
				70					75					
TAT	GAT	CCA	CTT	TTG	GAT	TCA	TCA	GAT	ATG	ACA	TTT	GAT	GAC	347
Tyr	Asp	Pro	Leu	Leu	Asp	Ser	Ser	Asp	Met	Thr	Phe	Asp	Asp	
80					85					90				
TGG	ATT	CAT	ATC	GGT	AAA	GAT	ATT	CAA	AGA	GCT	TAC	GAT	CAA	389
Trp	Ile	His	Ile	Gly	Lys	Asp	Ile	Gln	Arg	Ala	Tyr	Asp	Gln	
	95					100					105			
TAT	GTG	GGC	TTT	GTT	ATA	TTA	CAT	GGT	ACT	GAT	ACA	CTG	GCA	431
Tyr	Val	Gly	Phe	Val	Ile	Leu	His	Gly	Thr	Asp	Thr	Leu	Ala	
		110					115					120		
TAT	ACG	GC												439
Tyr	Thr													

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 123 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met	Gln	Cys	Glu	Glu	Ala	His	Val	Leu	Val	Leu	Tyr	Thr	Gly
1				5					10				
Gly	Thr	Ile	Gly	Met	Lys	Tyr	Ile	Asp	Gly	Val	Tyr	Gln	Pro
15				20					25				
Glu	Ala	Asn	Tyr	Leu	Leu	His	Ala	Ile	Arg	Asp	Leu	Ser	Leu
	30					35					40		
Leu	Asn	Asp	Asp	Asp	Tyr	Val	Ser	Thr	Tyr	Tyr	Ser	Asp	Ala
		45					50					55	
Glu	Ile	Arg	Pro	Tyr	Cys	Leu	Pro	Pro	Leu	Gln	His	Ser	Lys
			60					65					70
Lys	Arg	Val	Val	Tyr	Trp	Met	Ile	Glu	Tyr	Asp	Pro	Leu	Leu
				75					80				
Asp	Ser	Ser	Asp	Met	Thr	Phe	Asp	Asp	Trp	Ile	His	Ile	Gly
85					90					95			
Lys	Asp	Ile	Gln	Arg	Ala	Tyr	Asp	Gln	Tyr	Val	Gly	Phe	Val
	100					105					110		
Ile	Leu	His	Gly	Thr	Asp	Thr	Leu	Ala	Tyr	Thr			
		115					120						

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 439 nucleotides
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GCCGTATATG	CCAGTGTATC	AGTACCATGT	AATATAACAA	AGCCACATA	50
TTGATCGTAA	GCTCTTTGAA	TATCTTTACC	GATATGAATC	CAGTCATCAA	100
ATGTCATATC	TGATGAATCC	AAAAGTGGAT	CATATTCGAT	CATCCAATAA	150
ACAACACGTT	TTTTTGAATG	TTGTAGTGGT	GGCAAACAAT	ATGGCCTTAT	200

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TTCGGCGTCA	GAATAATATG	TGGACACATA	ATCATCATCG	TTTAATAGTG	250
ATAAATCACG	TATGGCATGC	AGAAGATAAT	TAGCTTCTGG	CTGATACACT	300
CCATCAATGT	ATTTTCATCCC	AATCGTTCCA	CCTGTATATA	GCACTAAAAC	350
ATGCGCTTCT	TCACACTGCA	TCGGACTGAA	TGATATTTGT	ATAAATACTT	400
CCAATCTAAT	TTAATTGCTC	AAACTTGGGT	AATTAAACC		439

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 369 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i x) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 1..369

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ATG	CAG	TGT	GAA	GAA	GCG	CAT	GTT	TTA	GTG	CTA	TAT	ACA	GGT	42
Met	Gln	Cys	Glu	Glu	Ala	His	Val	Leu	Val	Leu	Tyr	Thr	Gly	
1				5					10					
GGA	ACG	ATT	GGG	ATG	AAA	TAC	ATT	GAT	GGA	GTG	TAT	CAG	CCA	84
Gly	Thr	Ile	Gly	Met	Lys	Tyr	Ile	Asp	Gly	Val	Tyr	Gln	Pro	
15					20					25				
GAA	GCT	AAT	TAT	CTT	CTG	CAT	GCC	ATA	CGT	GAT	TTA	TCA	CTA	126
Glu	Ala	Asn	Tyr	Leu	Leu	His	Ala	Ile	Arg	Asp	Leu	Ser	Leu	
	30					35					40			
TTA	AAC	GAT	GAT	GAT	TAT	GTG	TCC	ACA	TAT	TAT	TCT	GAC	GCC	168
Leu	Asn	Asp	Asp	Asp	Tyr	Val	Ser	Thr	Tyr	Tyr	Ser	Asp	Ala	
		45					50					55		
GAA	ATA	AGG	CCA	TAT	TGT	TTG	CCA	CCA	CTA	CAA	CAT	TCA	AAA	210
Glu	Ile	Arg	Pro	Tyr	Cys	Leu	Pro	Pro	Leu	Gln	His	Ser	Lys	
			60				65						70	
AAA	CGT	GTT	GTT	TAT	TGG	ATG	ATC	GAA	TAT	GAT	CCA	CTT	TTG	252
Lys	Arg	Val	Val	Tyr	Trp	Met	Ile	Glu	Tyr	Asp	Pro	Leu	Leu	
				75					80					
GAT	TCA	TCA	GAT	ATG	ACA	TTT	GAT	GAC	TGG	ATT	CAT	ATC	GGT	294
Asp	Ser	Ser	Asp	Met	Thr	Phe	Asp	Asp	Trp	Ile	His	Ile	Gly	
	85				90					95				
AAA	GAT	ATT	CAA	AGA	GCT	TAC	GAT	CAA	TAT	GTG	GGC	TTT	GTT	336
Lys	Asp	Ile	Gln	Arg	Ala	Tyr	Asp	Gln	Tyr	Val	Gly	Phe	Val	
	100					105					110			
ATA	TTA	CAT	GGT	ACT	GAT	ACA	CTG	GCA	TAT	ACG				369
Ile	Leu	His	Gly	Thr	Asp	Thr	Leu	Ala	Tyr	Thr				
		115					120							

(2) INFORMATION FOR SEQ ID NO:10:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 369 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:10:

CGTATATGCC	AGTGTATCAG	TACCATGTAA	TATAACAAAG	CCCACATATT	50
GATCGTAAGC	TCTTTGAATA	TCTTTACCGA	TATGAATCCA	GTCATCAAAT	100

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GTCATATCTG	ATGAATCCAA	AAGTGGATCA	TATTCGATCA	TCCAATAAAC	150
AACACGTTTT	TTTGAATGTT	GTAGTGGTGG	CAAACAATAT	GGCCTTATTT	200
CGGCGTCAGA	ATAATATGTG	GACACATAAT	CATCATCGTT	TAATAGTGAT	250
AAATCACGTA	TGGCATGCAG	AAGATAATTA	GCTTCTGGCT	GATACACTCC	300
ATCAATGTAT	TTCATCCCAA	TCGTTCCACC	TGTATATAGC	ACTAAAACAT	350
GCGCTTCTTC	ACACTGCAT				369

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1770 nucleotides
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(i x) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..1770

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ATG	CAG	TGT	GAA	GAA	GCG	CAT	GTT	TTA	GTG	CTA	TAT	ACA	GGT	42
Met	Gln	Cys	Glu	Glu	Ala	His	Val	Leu	Val	Leu	Tyr	Thr	Gly	
1				5					10					
GGA	ACG	ATT	GGG	ATG	AAA	TAC	ATT	GAT	GGA	GTG	TAT	CAG	CCA	84
Gly	Thr	Ile	Gly	Met	Lys	Tyr	Ile	Asp	Gly	Val	Tyr	Gln	Pro	
15				20					25					
GAA	GCT	AAT	TAT	CTT	CTG	CAT	GCC	ATA	CGT	GAT	TTA	TCA	CTA	126
Glu	Ala	Asn	Tyr	Leu	Leu	His	Ala	Ile	Arg	Asp	Leu	Ser	Leu	
	30					35					40			
TTA	AAC	GAT	GAT	GAT	TAT	GTG	TCC	ACA	TAT	TAT	TCT	GAC	GCC	168
Leu	Asn	Asp	Asp	Asp	Tyr	Val	Ser	Thr	Tyr	Tyr	Ser	Asp	Ala	
		45				50						55		
GAA	ATA	AGG	CCA	TAT	TGT	TTG	CCA	CCA	CTA	CAA	CAT	TCA	AAA	210
Glu	Ile	Arg	Pro	Tyr	Cys	Leu	Pro	Pro	Leu	Gln	His	Ser	Lys	
			60					65					70	
AAA	CGT	GTT	GTT	TAT	TGG	ATG	ATC	GAA	TAT	GAT	CCA	CTT	TTG	252
Lys	Arg	Val	Val	Tyr	Trp	Met	Ile	Glu	Tyr	Asp	Pro	Leu	Leu	
				75				80						
GAT	TCA	TCA	GAT	ATG	ACA	TTT	GAT	GAC	TGG	ATT	CAT	ATC	GGT	294
Asp	Ser	Ser	Asp	Met	Thr	Phe	Asp	Asp	Trp	Ile	His	Ile	Gly	
85				90						95				
AAA	GAT	ATT	CAA	AGA	GCT	TAC	GAT	CAA	TAT	GTG	GGC	TTT	GTT	336
Lys	Asp	Ile	Gln	Arg	Ala	Tyr	Asp	Gln	Tyr	Val	Gly	Phe	Val	
100						105					110			
ATA	TTA	CAT	GGT	ACT	GAT	ACA	CTG	GCA	TAT	ACG	GCA	TGT	GCT	378
Ile	Leu	His	Gly	Thr	Asp	Thr	Leu	Ala	Tyr	Thr	Ala	Cys	Ala	
		115					120					125		
TTG	TCA	TTT	ATG	CTG	GAG	AAC	GTA	AGA	AAA	CCC	ATT	GTT	ATT	420
Leu	Ser	Phe	Met	Leu	Glu	Asn	Val	Arg	Lys	Pro	Ile	Val	Ile	
			130					135					140	
ACA	GGA	GCT	CAA	ATA	CCA	GTG	TGT	GAA	GTT	CGT	TCT	GAC	GGT	462
Thr	Gly	Ala	Gln	Ile	Pro	Val	Cys	Glu	Val	Arg	Ser	Asp	Gly	
				145					150					
CGA	GAA	AAT	TTG	ATT	GGT	GCA	CTG	ATT	ATT	GCA	GCC	AAT	TAT	504
Arg	Glu	Asn	Leu	Ile	Gly	Ala	Leu	Ile	Ile	Ala	Ala	Asn	Tyr	
155					160					165				
GAT	ATT	CCT	GAA	GTT	ACT	GTA	TAT	TTC	AAT	AAT	AAG	CTG	TTT	546
Asp	Ile	Pro	Glu	Val	Thr	Val	Tyr	Phe	Asn	Asn	Lys	Leu	Phe	

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170				175				180						
CGA	GGA	AAT	CGT	ACA	GTA	AAA	ATA	GAT	AAC	AGA	TCA	ATG	GAT	588
Arg	Gly	Asn	Arg	Thr	Val	Lys	Ile	Asp	Asn	Arg	Ser	Met	Asp	
		185					190					195		
GCT	TTT	GAA	AGT	CCA	AAT	ATG	CTT	CCA	ATT	GCT	TAC	ATG	GAT	630
Ala	Phe	Glu	Ser	Pro	Asn	Met	Leu	Pro	Ile	Ala	Tyr	Met	Asp	
			200					205					210	
GTT	GAT	ATA	AAA	GTT	AAT	TAT	GAT	TCA	ATA	TTT	CGT	TCA	CCA	672
Val	Asp	Ile	Lys	Val	Asn	Tyr	Asp	Ser	Ile	Phe	Arg	Ser	Pro	
				215						220				
TCA	ATG	GCT	CCA	TTC	GTA	GTA	CAC	GAC	CAA	TTA	TGT	CGA	AAT	714
Ser	Met	Ala	Pro	Phe	Val	Val	His	Asp	Gln	Leu	Cys	Arg	Asn	
					230					235				
GTT	GGA	TTG	TTG	AGA	ATT	TTT	CCA	TCG	ATG	TCT	ATA	GAA	AAC	756
Val	Gly	Leu	Leu	Arg	Ile	Phe	Pro	Ser	Met	Ser	Ile	Glu	Asn	
	240					245					250			
GTT	AGA	GCA	TCC	TTG	CAG	GCA	CCT	ATT	GAA	GGT	GTT	GTT	CTG	798
Val	Arg	Ala	Ser	Leu	Gln	Ala	Pro	Ile	Glu	Gly	Val	Val	Leu	
		255					260					265		
CAG	ACG	TTT	GGT	GCT	GGT	AAT	ATG	CCC	TCC	CAT	AGG	ACA	GAT	840
Gln	Thr	Phe	Gly	Ala	Gly	Asn	Met	Pro	Ser	His	Arg	Thr	Asp	
			270					275					280	
ATA	ATC	GAT	GAA	TTG	AAA	AAA	GCT	GTT	GAT	CGA	GGA	TGT	ATT	882
Ile	Ile	Asp	Glu	Leu	Lys	Lys	Ala	Val	Asp	Arg	Gly	Cys	Ile	
				285					290					
ATT	ATT	AAT	TGC	TCA	CAG	TGT	GTC	CGT	GGA	CAA	GTA	GAT	ATT	924
Ile	Ile	Asn	Cys	Ser	Gln	Cys	Val	Arg	Gly	Gln	Val	Asp	Ile	
					300					305				
CAT	TAT	TTA	ACG	GGA	AAG	GTT	CTA	TAC	GAC	ATG	GGA	ATT	ATT	966
His	Tyr	Leu	Thr	Gly	Lys	Val	Leu	Tyr	Asp	Met	Gly	Ile	Ile	
	310					315					320			
CCT	GGT	TCA	GAT	ATG	ACT	GCA	GAA	GCA	GCA	TTA	ACA	AAA	TTA	1008
Pro	Gly	Ser	Asp	Met	Thr	Ala	Glu	Ala	Ala	Leu	Thr	Lys	Leu	
		325					330					335		
TCG	TAT	GTA	TTG	AGC	AAA	GAT	TGT	TGG	GAA	CTT	GTG	GAG	AAA	1050
Ser	Tyr	Val	Leu	Ser	Lys	Asp	Cys	Trp	Glu	Leu	Val	Glu	Lys	
			340					345					350	
AAA	GCA	ATG	ATG	GTT	AAA	AAT	ATC	AGA	GGC	GAA	TTA	ACT	GTT	1092
Lys	Ala	Met	Met	Val	Lys	Asn	Ile	Arg	Gly	Glu	Leu	Thr	Val	
				355					360					
GCA	AAA	GCA	GAA	CCA	CTC	AAA	GAT	CTA	GAA	ATC	GTA	TCA	CAG	1134
Ala	Lys	Ala	Glu	Pro	Leu	Lys	Asp	Leu	Glu	Ile	Val	Ser	Gln	
					370					375				
ATG	GCA	AGA	TTC	CTG	CAT	CTA	AGT	TCT	TCT	CAT	GAA	ATG	AAA	1176
Met	Ala	Arg	Phe	Leu	His	Leu	Ser	Ser	Ser	His	Glu	Met	Lys	
	380					385					390			
CTC	CTC	TGT	CAT	GCT	ATT	TTT	CCA	CAA	TTA	TTG	TGT	TAT	GCA	1218
Leu	Leu	Cys	His	Ala	Ile	Phe	Pro	Gln	Leu	Leu	Cys	Tyr	Ala	
		395					400				405			
GCT	AGT	AAT	GGG	GAT	ATC	GAA	ATG	CTA	AAG	GCA	CTT	CAT	GAA	1260
Ala	Ser	Asn	Gly	Asp	Ile	Glu	Met	Leu	Lys	Ala	Leu	His	Glu	
			410					415					420	
AAT	GGA	GTT	GAT	CTT	TCG	GTT	GTT	GAC	TAT	AAT	GGA	CGC	AAT	1302
Asn	Gly	Val	Asp	Leu	Ser	Val	Val	Asp	Tyr	Asn	Gly	Arg	Asn	
				425				430						
GCT	TTG	CAT	GTA	GCA	GCG	AGT	GCA	GGT	CAC	GTT	GGT	GCT	GTC	1344
Ala	Leu	His	Val	Ala	Ala	Ser	Ala	Gly	His	Val	Gly	Ala	Val	
					440					445				
AAA	TAT	CTG	TTG	ACC	CAA	GGT	GTT	AGT	TTT	CAT	CTG	AGA	GAT	1386
Lys	Tyr	Leu	Leu	Thr	Gln	Gly	Val	Ser	Phe	His	Leu	Arg	Asp	

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450	455	460	
CAA TGG GAT GAG AAT GCC CTC GTA AGT GCA GTA AAA ATG AAA Gln Trp Asp Glu Asn Ala Leu Val Ser Ala Val Lys Met Lys 465 470 475			1428
AAT AAG ATC TTA ATT GAA ACT TTG CGA TCT GCA GGG GCA CTG Asn Lys Ile Leu Ile Glu Thr Leu Arg Ser Ala Gly Ala Leu 480 485 490			1470
CTT TCC ATA AAT TCA CGC AGA TTA GGT GTT GAA CTA TGT CTA Leu Ser Ile Asn Ser Arg Arg Leu Gly Val Glu Leu Cys Leu 495 500			1512
TGT GCC AGC TAT GGC GAC ACG GAA ACA CTG AAT TCT TGG CTT Cys Ala Ser Tyr Gly Asp Thr Glu Thr Leu Asn Ser Trp Leu 505 510 515			1554
GCT GCC GGA GCC GAT ATA AAT CAA CAA GAT TAC AAT GGC GAA Ala Ala Gly Ala Asp Ile Asn Gln Gln Asp Tyr Asn Gly Glu 520 525 530			1596
ACT GCT TTG CAT ATT GCG GTG AAA TCG AGA AAT AAG CAA TTG Thr Ala Leu His Ile Ala Val Lys Ser Arg Asn Lys Gln Leu 535 540 545			1638
GTA CAT TAT TTG CTG GAT AGA GAT GCA GAT CCA TAC AAA ATT Val His Tyr Leu Leu Asp Arg Asp Ala Asp Pro Tyr Lys Ile 550 555 560			1680
GAC GAT TTT AAT TTA ACG CCT CTT AGA CAT GCT AAA AAA CTT Asp Asp Phe Asn Leu Thr Pro Leu Arg His Ala Lys Lys Leu 565 570			1722
AAT TTA CAA GAT CTA GTC ATT AGA ATG AAA AAG ATG AAA AAA Asn Leu Gln Asp Leu Val Ile Arg Met Lys Lys Met Lys Lys 575 580 585			1764
GTT CAG Val Gln 590			1770

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 590 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Protein

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Gln Cys Glu Glu Ala His Val Leu Val Leu Tyr Thr Gly
1 5 10

Gly Thr Ile Gly Met Lys Tyr Ile Asp Gly Val Tyr Gln Pro
15 20 25

Glu Ala Asn Tyr Leu Leu His Ala Ile Arg Asp Leu Ser Leu
30 35 40

Leu Asn Asp Asp Asp Tyr Val Ser Thr Tyr Tyr Ser Asp Ala
45 50 55

Glu Ile Arg Pro Tyr Cys Leu Pro Pro Leu Gln His Ser Lys
60 65 70

Lys Arg Val Val Tyr Trp Met Ile Glu Tyr Asp Pro Leu Leu
75 80

Asp Ser Ser Asp Met Thr Phe Asp Asp Trp Ile His Ile Gly
85 90 95

Lys Asp Ile Gln Arg Ala Tyr Asp Gln Tyr Val Gly Phe Val
100 105 110

Ile Leu His Gly Thr Asp Thr Leu Ala Tyr Thr Ala Cys Ala
115 120 125

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Leu	Ser	Phe	Met 130	Leu	Glu	Asn	Val	Arg 135	Lys	Pro	Ile	Val	Ile 140
Thr	Gly	Ala	Gln 145	Ile	Pro	Val	Cys	Glu	Val 150	Arg	Ser	Asp	Gly
Arg 155	Glu	Asn	Leu	Ile	Gly 160	Ala	Leu	Ile	Ile	Ala 165	Ala	Asn	Tyr
Asp 170	Ile	Pro	Glu	Val	Thr	Val 175	Tyr	Phe	Asn	Asn	Lys 180	Leu	Phe
Arg	Gly	Asn 185	Arg	Thr	Val	Lys	Ile 190	Asp	Asn	Arg	Ser	Met 195	Asp
Ala	Phe	Glu	Ser 200	Pro	Asn	Met	Leu	Pro 205	Ile	Ala	Tyr	Met	Asp 210
Val	Asp	Ile	Lys 215	Val	Asn	Tyr	Asp	Ser	Ile 220	Phe	Arg	Ser	Pro
Ser 225	Met	Ala	Pro	Phe	Val 230	Val	His	Asp	Gln	Leu 235	Cys	Arg	Asn
Val	Gly 240	Leu	Leu	Arg	Ile	Phe 245	Pro	Ser	Met	Ser	Ile 250	Glu	Asn
Val	Arg	Ala 255	Ser	Leu	Gln	Ala	Pro 260	Ile	Glu	Gly	Val	Val 265	Leu
Gln	Thr	Phe	Gly 270	Ala	Gly	Asn	Met	Pro 275	Ser	His	Arg	Thr	Asp 280
Ile	Ile	Asp	Glu	Leu 285	Lys	Lys	Ala	Val	Asp 290	Arg	Gly	Cys	Ile
Ile 295	Ile	Asn	Cys	Ser	Gln 300	Cys	Val	Arg	Gly	Gln 305	Val	Asp	Ile
His	Tyr 310	Leu	Thr	Gly	Lys	Val 315	Leu	Tyr	Asp	Met	Gly 320	Ile	Ile
Pro	Gly	Ser 325	Asp	Met	Thr	Ala	Glu 330	Ala	Ala	Leu	Thr	Lys 335	Leu
Ser	Tyr	Val	Leu 340	Ser	Lys	Asp	Cys	Trp 345	Glu	Leu	Val	Glu	Lys 350
Lys	Ala	Met	Met 355	Val	Lys	Asn	Ile	Arg	Gly 360	Glu	Leu	Thr	Val
Ala 365	Lys	Ala	Glu	Pro	Leu 370	Lys	Asp	Leu	Glu	Ile 375	Val	Ser	Gln
Met	Ala 380	Arg	Phe	Leu	His	Leu 385	Ser	Ser	Ser	His	Glu 390	Met	Lys
Leu	Leu	Cys 395	His	Ala	Ile	Phe	Pro 400	Gln	Leu	Leu	Cys	Tyr 405	Ala
Ala	Ser	Asn	Gly 410	Asp	Ile	Glu	Met	Leu 415	Lys	Ala	Leu	His	Glu 420
Asn	Gly	Val	Asp 425	Leu	Ser	Val	Val	Asp	Tyr 430	Asn	Gly	Arg	Asn
Ala 435	Leu	His	Val	Ala	Ala 440	Ser	Ala	Gly	His	Val 445	Gly	Ala	Val
Lys	Tyr 450	Leu	Leu	Thr	Gln	Gly 455	Val	Ser	Phe	His	Leu 460	Arg	Asp
Gln	Trp	Asp 465	Glu	Asn	Ala	Leu	Val 470	Ser	Ala	Val	Lys	Met 475	Lys
Asn	Lys	Ile 480	Leu	Ile	Glu	Thr	Leu	Arg 485	Ser	Ala	Gly	Ala	Leu 490
Leu	Ser	Ile	Asn	Ser	Arg	Arg	Leu	Gly	Val	Glu	Leu	Cys	Leu

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495					500								
Cys 505	Ala	Ser	Tyr	Gly	Asp 510	Thr	Glu	Thr	Leu	Asn 515	Ser	Trp	Leu
Ala 520	Ala	Gly	Ala	Asp	Ile	Asn 525	Gln	Gln	Asp	Tyr	Asn 530	Gly	Glu
Thr	Ala	Leu 535	His	Ile	Ala	Val	Lys 540	Ser	Arg	Asn	Lys	Gln 545	Leu
Val	His	Tyr	Leu 550	Leu	Asp	Arg	Asp	Ala 555	Asp	Pro	Tyr	Lys	Ile 560
Asp	Asp	Phe	Asn 565	Leu	Thr	Pro	Leu	Arg	His 570	Ala	Lys	Lys	Leu
Asn 575	Leu	Gln	Asp	Leu	Val 580	Ile	Arg	Met	Lys	Lys 585	Met	Lys	Lys
Val	Gln 590												

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1770 nucleotides
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```

CTGAACTTTT TTCATCTTTT TCATTCTAAT GACTAGATCT TGTA AATTAA      50
GTTTTTTAGC ATGTCTAAGA GGC GTTAAAT TAAAATCGTC AATTTTGTAT      100
GGATCTGCAT CTCTATCCAG CAAATAATGT ACCAATTGCT TATTTCTCGA      150
TTTCACCGCA ATATGCAAAG CAGTTTCGCC ATTGTAATCT TGTTGATTTA      200
TATCGGCTCC GGCAGCAAGC CAAGAATTCA GTGTTTCCGT GTCGCCATAG      250
CTGGCACATA GACATAGTTC AACACCTAAT CTGCGTGAAT TTATGGAAAG      300
CAGTGCCCCCT GCAGATCGCA AAGTTTCAAT TAAGATCTTA TTTTTCATTT      350
T TACTGCACT TACGAGGGCA TTCTCATCCC ATTGATCTCT CAGATGAAAA      400
CTAACACCTT GGGTCAACAG ATATTTGACA GCACCAACGT GACCTGCACT      450
CGCTGCTACA TGCAAAGCAT TGC GTCCATT ATAGTCAACA ACCGAAAGAT      500
CAACTCCATT TTCATGAAGT GCCTTTAGCA TTTCGATATC CCCATTACTA      550
GCTGCATAAC ACAATAATTG TGGAAAAATA GCATGACAGA GGAGTTTCAT      600
TTCATGAGAA GAACTTAGAT GCAGGAATCT TGCCATCTGT GATACGATTT      650
CTAGATCTTT GAGTGGTTCT GCTTTTGCAA CAGTTAATTC GCCTCTGATA      700
TTTTTAACCA TCATTGCTTT TTTCTCCACA AGTTCCCAAC AATCTTTGCT      750
CAATACATAC GATAATTTTG TTAATGCTGC TTCTGCAGTC ATATCTGAAC      800
CAGGAATAAT TCCCATGTGC TATAGAACCT TTCCCGTTAA ATAATGAATA      850
TCTACTTGTC CACGGACACA CTGTGAGCAA TTAATAATAA TACATCCTCG      900
ATCAACAGCT TTTTTC AATT CATCGATTAT ATCTGTCCTA TGGGAGGGCA      950
TATTACCAGC ACCAAACGTC TGCAGAACAA CACCTTCAAT AGGTGCCTGC     1000
AAGGATGCTC TAACGTTTTC TATAGACATC GATGGAAAAA TTCTCAACAA     1050
TCCAACATTT CGACATAATT GGTCTGTGAC TACGAATGGA GCCATTGATG     1100

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GTGAACGAAA	TATTGAATCA	TAATTAACCT	TTATATCAAC	ATCCATGTAA	1 1 5 0
GCAATTGGAA	GCATATTTGG	ACTTTCAAAA	GCATCCATTG	ATCTGTTATC	1 2 0 0
TATTTTACT	GTACGATTTT	CTCGAAACAG	CTTATTATTG	AAATATACAG	1 2 5 0
TAACTTCAGG	AATATCATAA	TTGGCTGCAA	TAATCAGTGC	ACCAATCAAA	1 3 0 0
TTTTCTCGAC	CGTCAGAACG	AACTTCACAC	ACTGGTATTT	GAGCTCCTGT	1 3 5 0
AATAACAATG	GGTTTTCTTA	CGTTCTCCAG	CATAAATGAC	AAAGCACATG	1 4 0 0
CCGTATATGC	CAGTGTATCA	GTACCATGTA	ATATAACAAA	GCCCACATAT	1 4 5 0
TGATCGTAAG	CTCTTTGAAT	ATCTTTACCG	ATATGAATCC	AGTCATCAAA	1 5 0 0
TGTCATATCT	GATGAATCCA	AAAGTGGATC	ATATTCGATC	ATCCAATAAA	1 5 5 0
CAACACGTTT	TTTTGAATGT	TGTAGTGGTG	GCAAACAATA	TGGCCTTATT	1 6 0 0
TCGGCGTCAG	AATAATATGT	GGACACATAA	TCATCATCGT	TTAATAGTGA	1 6 5 0
TAAATCACGT	ATGGCATGCA	GAAGATAATT	AGCTTCTGGC	TGATACACTC	1 7 0 0
CATCAATGTA	TTTCATCCCA	ATCGTTCCAC	CTGTATATAG	CACTAAAACA	1 7 5 0
TGCGCTTCTT	CACACTGCAT				1 7 7 0

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 nucleotides
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Primer

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:14:

AATTAACCCT CACTAAAGGG 2 0

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 nucleotides
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Primer

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:15:

GGAAACAGCT ATGACCATG 1 9

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 nucleotides
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Primer

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CGCTCTAGAA CTAGTGGATC 2 0

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 nucleotides
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single

-continued

(D) TOPOLOGY: linear

(i i) MOLECULE TYPE:

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:17:

G T A A T A C G A C T C A C T A T A G G G C

2 2

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 nucleotides

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Primer

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:18:

G T A A A A C G A C G G C C A G T

1 7

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 nucleotides

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Primer

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:19:

C A A T A T T T C G T T C A C C A T C A A T G G C

2 5

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 nucleotides

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Primer

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:20:

C G G C T C C G G C A G C A A G C C A A G A A T T C

2 6

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 nucleotides

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Primer

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:21:

G G T T T A A T T A C C C A A G T T T G A G

2 2

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 nucleotides

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(i i) MOLECULE TYPE: Primer

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:22:

-continued

80				85				90						
TGG	ATT	CAT	ATC	GGT	AAA	GAT	ATT	CAA	AGA	GCT	TAC	GAT	CAA	389
Trp	Ile	His	Ile	Gly	Lys	Asp	Ile	Gln	Arg	Ala	Tyr	Asp	Gln	
	95					100					105			
TAT	GTG	GGC	TTT	GTT	ATA	TTA	CAT	GGT	ACT	GAT	ACA	CTG	GCA	431
Tyr	Val	Gly	Phe	Val	Ile	Leu	His	Gly	Thr	Asp	Thr	Leu	Ala	
		110					115					120		
TAT	ACG	GCA	TGT	GCT	TTG	TCA	TTT	ATG	CTG	GAG	AAC	GTA	AGA	473
Tyr	Thr	Ala	Cys	Ala	Leu	Ser	Phe	Met	Leu	Glu	Asn	Val	Arg	
			125					130					135	
AAA	CCC	ATT	GTT	ATT	ACA	GGA	GCT	CAA	ATA	CCA	GTG	TGT	GAA	515
Lys	Pro	Ile	Val	Ile	Thr	Gly	Ala	Gln	Ile	Pro	Val	Cys	Glu	
				140						145				
GTT	CGT	TCT	GAC	GGT	CGA	GAA	AAT	TTG	ATT	GGT	GCA	CTG	ATT	557
Val	Arg	Ser	Asp	Gly	Arg	Glu	Asn	Leu	Ile	Gly	Ala	Leu	Ile	
					155					160				
ATT	GCA	GCC	AAT	TAT	GAT	ATT	CCT	GAA	GTT	ACT	GTA	TAT	TTC	599
Ile	Ala	Ala	Asn	Tyr	Asp	Ile	Pro	Glu	Val	Thr	Val	Tyr	Phe	
	165					170					175			
AAT	AAT	AAG	CTG	TTT	CGA	GGA	AAT	CGT	ACA	GTA	AAA	ATA	GAT	641
Asn	Asn	Lys	Leu	Phe	Arg	Gly	Asn	Arg	Thr	Val	Lys	Ile	Asp	
		180					185					190		
AAC	AGA	TCA	ATG	GAT	GCT	TTT	GAA	AGT	CCA	AAT	ATG	CTT	CCA	683
Asn	Arg	Ser	Met	Asp	Ala	Phe	Glu	Ser	Pro	Asn	Met	Leu	Pro	
			195					200					205	
ATT	GCT	TAC	ATG	GAT	GTT	GAT	ATA	AAA	GTT	AAT	TAT	GAT	TCA	725
Ile	Ala	Tyr	Met	Asp	Val	Asp	Ile	Lys	Val	Asn	Tyr	Asp	Ser	
				210					215					
ATA	TTT	CGT	TCA	CCA	TCA	ATG	GCT	CCA	TTC	GTA	GTA	CAC	GAC	767
Ile	Phe	Arg	Ser	Pro	Ser	Met	Ala	Pro	Phe	Val	Val	His	Asp	
					225					230				
CAA	TTA	TGT	CGA	AAT	GTT	GGA	TTG	TTG	AGA	ATT	TTT	CCA	TCG	809
Gln	Leu	Cys	Arg	Asn	Val	Gly	Leu	Leu	Arg	Ile	Phe	Pro	Ser	
	235					240					245			
ATG	TCT	ATA	GAA	AAC	GTT	AGA	GCA	TCC	TTG	CAG	GCA	CCT	ATT	851
Met	Ser	Ile	Glu	Asn	Val	Arg	Ala	Ser	Leu	Gln	Ala	Pro	Ile	
		250					255					260		
GAA	GGT	GTT	GTT	CTG	CAG	ACG	TTT	GGT	GCT	GGT	AAT	ATG	CCC	893
Glu	Gly	Val	Val	Leu	Gln	Thr	Phe	Gly	Ala	Gly	Asn	Met	Pro	
			265					270				275		
TCC	CAT	AGG	ACA	GAT	ATA	ATC	GAT	GAA	TTG	AAA	AAA	GCT	GTT	935
Ser	His	Arg	Thr	Asp	Ile	Ile	Asp	Glu	Leu	Lys	Lys	Ala	Val	
				280					285					
GAT	CGA	GGA	TGT	ATT	ATT	ATT	AAT	TGC	TCA	CAG	TGT	GTC	CGT	977
Asp	Arg	Gly	Cys	Ile	Ile	Ile	Asn	Cys	Ser	Gln	Cys	Val	Arg	
				290		295				300				
GGA	CAA	GTA	GAT	ATT	CAT	TAT	TTA	ACG	GGA	AAG	GTT	CTA	TAC	1019
Gly	Gln	Val	Asp	Ile	His	Tyr	Leu	Thr	Gly	Lys	Val	Leu	Tyr	
	305					310					315			
GAC	ATG	GGA	ATT	ATT	CCT	GGT	TCA	GAT	ATG	ACT	GCA	GAA	GCA	1061
Asp	Met	Gly	Ile	Ile	Pro	Gly	Ser	Asp	Met	Thr	Ala	Glu	Ala	
		320					325					330		
GCA	TTA	ACA	AAA	TTA	TCG	TAT	GTA	TTG	AGC	AAA	GAT	TGT	TGG	1103
Ala	Leu	Thr	Lys	Leu	Ser	Tyr	Val	Leu	Ser	Lys	Asp	Cys	Trp	
			335					340					345	
GAA	CTT	GTG	GAG	AAA	AAA	GCA	ATG	ATG	GTT	AAA	AAT	ATC	AGA	1145
Glu	Leu	Val	Glu	Lys	Lys	Ala	Met	Met	Val	Lys	Asn	Ile	Arg	
				350					355					
GGC	GAA	TTA	ACT	GTT	GCA	AAA	GCA	GAA	CCA	CTC	AAA	GAT	CTA	1187
Gly	Glu	Leu	Thr	Val	Ala	Lys	Ala	Glu	Pro	Leu	Lys	Asp	Leu	

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360				365				370						
GAA	ATC	GTA	TCA	CAG	ATG	GCA	AGA	TTC	CTG	CAT	CTA	AGT	TCT	1229
Glu	Ile	Val	Ser	Gln	Met	Ala	Arg	Phe	Leu	His	Leu	Ser	Ser	
	375					380					385			
TCT	CAT	GAA	ATG	AAA	CTC	CTC	TGT	CAT	GCT	ATT	TTT	CCA	CAA	1271
Ser	His	Glu	Met	Lys	Leu	Leu	Cys	His	Ala	Ile	Phe	Pro	Gln	
		390					395					400		
TTA	TTG	TGT	TAT	GCA	GCT	AGT	AAT	GGG	GAT	ATC	GAA	ATG	CTA	1313
Leu	Leu	Cys	Tyr	Ala	Ala	Ser	Asn	Gly	Asp	Ile	Glu	Met	Leu	
			405					410					415	
AAG	GCA	CTT	CAT	GAA	AAT	GGA	GTT	GAT	CTT	TCG	GTT	GTT	GAC	1355
Lys	Ala	Leu	His	Glu	Asn	Gly	Val	Asp	Leu	Ser	Val	Val	Asp	
				420					425					
TAT	AAT	GGA	CGC	AAT	GCT	TTG	CAT	GTA	GCA	GCG	AGT	GCA	GGT	1397
Tyr	Asn	Gly	Arg	Asn	Ala	Leu	His	Val	Ala	Ala	Ser	Ala	Gly	
					435						440			
CAC	GTT	GGT	GCT	GTC	AAA	TAT	CTG	TTG	ACC	CAA	GGT	GTT	AGT	1439
His	Val	Gly	Ala	Val	Lys	Tyr	Leu	Leu	Thr	Gln	Gly	Val	Ser	
	445					450					455			
TTT	CAT	CTG	AGA	GAT	CAA	TGG	GAT	GAG	AAT	GCC	CTC	GTA	AGT	1481
Phe	His	Leu	Arg	Asp	Gln	Trp	Asp	Glu	Asn	Ala	Leu	Val	Ser	
		460					465					470		
GCA	GTA	AAA	ATG	AAA	AAT	AAG	ATC	TTA	ATT	GAA	ACT	TTG	CGA	1523
Ala	Val	Lys	Met	Lys	Asn	Lys	Ile	Leu	Ile	Glu	Thr	Leu	Arg	
			475					480					485	
TCT	GCA	GGG	GCA	CTG	CTT	TCC	ATA	AAT	TCA	CGC	AGA	TTA	GGT	1565
Ser	Ala	Gly	Ala	Leu	Leu	Ser	Ile	Asn	Ser	Arg	Arg	Leu	Gly	
				490					495					
GTT	GAA	CTA	TGT	CTA	TGT	GCC	AGC	TAT	GGC	GAC	ACG	GAA	ACA	1607
Val	Glu	Leu	Cys	Leu	Cys	Ala	Ser	Tyr	Gly	Asp	Thr	Glu	Thr	
	500				505					510				
CTG	AAT	TCT	TGG	CTT	GCT	GCC	GGA	GCC	GAT	ATA	AAT	CAA	CAA	1649
Leu	Asn	Ser	Trp	Leu	Ala	Ala	Gly	Ala	Asp	Ile	Asn	Gln	Gln	
	515					520					525			
GAT	TAC	AAT	GGC	GAA	ACT	GCT	TTG	CAT	ATT	GCG	GTG	AAA	TCG	1691
Asp	Tyr	Asn	Gly	Glu	Thr	Ala	Leu	His	Ile	Ala	Val	Lys	Ser	
		530					535					540		
AGA	AAT	AAG	CAA	TTG	GTA	CAT	TAT	TTG	CTG	GAT	AGA	GAT	GCA	1733
Arg	Asn	Lys	Gln	Leu	Val	His	Tyr	Leu	Leu	Asp	Arg	Asp	Ala	
			545					550					555	
GAT	CCA	TAC	AAA	ATT	GAC	GAT	TTT	AAT	TTA	ACG	CCT	CTT	AGA	1775
Asp	Pro	Tyr	Lys	Ile	Asp	Asp	Phe	Asn	Leu	Thr	Pro	Leu	Arg	
				560					565					
CAT	GCT	AAA	AAA	CTT	AAT	TTA	CAA	GAT	CTA	GTC	ATT	AGA	ATG	1817
His	Ala	Lys	Lys	Leu	Asn	Leu	Gln	Asp	Leu	Val	Ile	Arg	Met	
	570				575					580				
AAA	AAG	ATG	AAA	AAA	GTT	CAG	TAA	TGTTGCTGCA	GAAAATAAAG				1861	
Lys	Lys	Met	Lys	Lys	Val	Gln								
	585					590								
ATCTTATGCA CTCAGAATGT ATTCAGAAGT ATGGTACAAA AGCCTTAAAT													1911	
TATGCTAGAT CTTGCATGAT TTCTAGCTTT TTAAATGGTA ATTTTTGTTC													1961	
CGTCTTTTTT CGCAAAGACT GATATAATTT AATGAAAAAA AACCTTGTTT													2011	
ATTCATCGAT TCCTTTTTTA AACAAAATAG TATTTAATGG CTAAAAAAA													2061	
AAAAAAAAAA AA													2073	

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

-continued

(A) LENGTH: 2073 nucleotides
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA

(x i) SEQUENCE DESCRIPTION: SEQ ID NO:26:

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TTTTTTTTTT TTTTTTTTTT AGCCATTAAA TACTATTTTG TTTAAAAAAG      5 0
GAATCGATGA ATAAACAAGG TTTTTTTTCA TTAAATTATA TCAGTCTTTG      1 0 0
CGAAAAAAGA CGGAACAAAA ATTACCATTT AAAAAGCTAG AAATCATGCA      1 5 0
AGATCTAGCA TAATTTAAGG CTTTTGTACC ATACTTCTGA ATACATTCTG      2 0 0
AGTGCATAAG ATCTTTATTT TCTGCAGCAA CATTACTGAA CTTTTTTCAT      2 5 0
CTTTTTTCATT CTAATGACTA GATCTTGTA AATTAAGTTTT TTAGCATGTC      3 0 0
TAAGAGGCGT TAAATTAAAA TCGTCAATTT TGTATGGATC TGCATCTCTA      3 5 0
TCCAGCAAAT AATGTACCAA TTGCTTATTT CTCGATTTCA CCGCAATATG      4 0 0
CAAAGCAGTT TCGCCATTGT AATCTTGTTG ATTTATATCG GCTCCGGCAG      4 5 0
CAAGCCAAGA ATTCAGTGTT TCCGTGTCGC CATAGCTGGC ACATAGACAT      5 0 0
AGTTCAACAC CTAATCTGCG TGAATTTATG GAAAGCAGTG CCCCTGCAGA      5 5 0
TCGCAAAGTT TCAATTAAGA TCTTATTTTT CATTTTTACT GCACTTACGA      6 0 0
GGGCATTCTC ATCCCATTTGA TCTCTCAGAT GAAAACCTAAC ACCTTGGGTC      6 5 0
AACAGATATT TGACAGCACC AACGTGACCT GCACTCGCTG CTACATGCAA      7 0 0
AGCATTGCGT CCATTATAGT CAACAACCGA AAGATCAACT CCATTTTCAT      7 5 0
GAAGTGCCTT TAGCATTTCG ATATCCCCAT TACTAGCTGC ATAACACAAT      8 0 0
AATTGTGGAA AAATAGCATG ACAGAGGAGT TTCATTTTCAT GAGAAGAAGT      8 5 0
TAGATGCAGG AATCTTGCCA TCTGTGATAC GATTTCTAGA TCTTTGAGTG      9 0 0
GTTCTGCTTT TGCAACAGTT AATTCGCCTC TGATATTTTT AACCATCATT      9 5 0
GCTTTTTTCT CCACAAGTTC CCAACAATCT TTGCTCAATA CATACGATAA     1 0 0 0
TTTTGTTAAT GCTGCTTCTG CAGTCATATC TGAACCAGGA ATAATTCCCA     1 0 5 0
TGTCGTATAG AACCTTTCCC GTTAAATAAT GAATATCTAC TTGTCCACGG     1 1 0 0
ACACACTGTG AGCAATTAAT AATAATACAT CCTCGATCAA CAGCTTTTTT     1 1 5 0
CAATTCATCG ATTATATCTG TCCTATGGGA GGGCATATTA CCAGCACCAA     1 2 0 0
ACGTCTGCAG AACAACACCT TCAATAGGTG CCTGCAAGGA TGCTCTAACG     1 2 5 0
TTTTCTATAG ACATCGATGG AAAAATTCTC AACAAATCCAA CATTTCGACA     1 3 0 0
TAATTGGTCG TGTACTACGA ATGGAGCCAT TGATGGTGAA CGAAATATTG     1 3 5 0
AATCATAATT AACTTTTATA TCAACATCCA TGTAAGCAAT TGGAAGCATA     1 4 0 0
TTTGGACTTT CAAAAGCATC CATTGATCTG TTATCTATTT TTAAGTACG     1 4 5 0
ATTTCTCTCG AACAGCTTAT TATTGAAATA TACAGTAACT TCAGGAATAT     1 5 0 0
CATAATTGGC TGCAATAATC AGTGCACCAA TCAAATTTTC TCGACCGTCA     1 5 5 0
GAACGAACTT CACACACTGG TATTTGAGCT CCTGTAATAA CAATGGGTTT     1 6 0 0
TCTTACGTTT TCCAGCATAA ATGACAAAGC ACATGCCGTA TATGCCAGTG     1 6 5 0
TATCAGTACC ATGTAATATA ACAAAGCCCA CATATTGATC GTAAGCTCTT     1 7 0 0
TGAATATCTT TACCGATATG AATCCAGTCA TCAAATGTCA TATCTGATGA     1 7 5 0
ATCCAAAAGT GGATCATATT CGATCATCCA ATAAACAACA CGTTTTTTTG     1 8 0 0

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AATGTTGTAG	TGGTGGCAAA	CAATATGGCC	TTATTTTCGGC	GTCAGAATAA	1 8 5 0
TATGTGGACA	CATAATCATC	ATCGTTTAAT	AGTGATAAAT	CACGTATGGC	1 9 0 0
ATGCAGAAGA	TAATTAGCTT	CTGGCTGATA	CACTCCATCA	ATGTATTTCA	1 9 5 0
TCCCAATCGT	TCCACCTGTA	TATAGCACTA	AAACATGCGC	TTCTTCACAC	2 0 0 0
TGCATCGGAC	TGAATGATAT	TTGTATAAAT	ACTTCCAATC	TAATTTAATT	2 0 5 0
GCTCAAACCTT	GGGTAATTAA	ACC			2 0 7 3

What is claimed is:

1. An isolated nucleic acid molecule comprising a nucleic acid sequence encoding a *Dirofilaria immitis* asparaginase protein, or its complementary sequence.

2. The nucleic acid molecule of claim 1, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of nDiASNase₁₇₅₃ (SEQ ID NO:1 and its complementary sequence), nDiASNase₁₅₁₈ (SEQ ID NO:4 and its complementary sequence), nDiASNase₄₃₉ (SEQ ID NO:6 and its complementary sequence), nDiASNase₃₆₉ (SEQ ID NO:9 and its complementary sequence), nDiASNase₁₇₇₀ (SEQ ID NO:11 and its complementary sequence) and nDiASNase₂₀₇₃ (SEQ ID NO:25 and its complementary sequence).

3. The nucleic acid molecule of claim 1, wherein said nucleic acid molecule comprises a nucleic acid sequence selected from the group consisting of: a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:25 and SEQ ID NO:26; and an allelic variant thereof.

4. The nucleic acid molecule of claim 1, wherein said nucleic acid molecule encodes a protein having an amino

15 acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:12.

5. The nucleic acid molecule of claim 1, wherein said nucleic acid molecule comprises at least about 15 contiguous nucleotides of a nucleic acid molecule having a nucleic acid sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:25 and SEQ ID NO:26.

6. A recombinant molecule comprising a nucleic acid molecule as set forth in claim 1 operatively linked to a transcription control sequence.

7. A recombinant virus comprising a nucleic acid molecule as set forth in claim 1.

8. A recombinant cell comprising a nucleic acid molecule as set forth in claim 1.

9. A method to produce a *Dirofilaria immitis* asparaginase protein comprising culturing in an effective medium a recombinant cell as set forth in claim 8.

* * * * *